# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

## PCT

### NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>21 June 2001 (21.06.01) | |
| **International application No.**<br>PCT/GB00/03301 | **Applicant's or agent's file reference**<br>SCE/BP/4929-WO |
| **International filing date** (day/month/year)<br>30 August 2000 (30.08.00) | **Priority date** (day/month/year)<br>31 August 1999 (31.08.99) |
| **Applicant**<br>SMITH, Clifford et al | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

26 March 2001 (26.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland** | Authorized officer<br>Olivia TEFY |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

Copy for the Elected Office (EO/US)

PCT/GB00/03301

# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

## PCT

### NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

HAMMER, Catriona, MacLeod
Amersham plc
Amersham Laboratories
White Lion Road
Amersham, Buckinghamshire HP7 9LL
ROYAUME-UNI

**Date of mailing** (day/month/year)
28 January 2002 (28.01.02)

**Applicant's or agent's file reference**
PA9943-PCT

**IMPORTANT NOTIFICATION**

**International application No.**
PCT/GB00/03301

**International filing date** (day/month/year)
30 August 2000 (30.08.00)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
EASTWOOD, Simon, Christopher
Stevens Hewlett & Perkins
1 St Augustine's Place
Bristol BS1 4UD
United Kingdom

State of Nationality:
State of Residence:
Telephone No.: +44 0 117 9226007
Facsimile No.: +44 0 117 9226009
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
HAMMER, Catriona, MacLeod
Amersham plc
Amersham Laboratories
White Lion Road
Amersham, Buckinghamshire HP7 9LL
United Kingdom

State of Nationality:
State of Residence:
Telephone No.: +44 1494 543982
Facsimile No.: +44 1494 543977
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

**The International Bureau of WIPO**
**34, chemin des Colombettes**
**1211 Geneva 20, Switzerland**

Facsimile No.: (41-22) 740.14.35

Authorized officer
R. Chrem

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004624287

# PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

# PCT

To:
STEVENS HEWLETT & PERKINS
Attn. EASTWOOD, S.CH.
1 St. Augustine's Place
Bristol BS1 4UD
UNITED KINGDOM

STEVENS HEWLETT & PERKINS BRISTOL
28 MAR 2001
12/4/01

## INVITATION TO PAY ADDITIONAL FEES

(PCT Article 17(3)(a) and Rule 40.1)

| | |
|---|---|
| Applicant's or agent's file reference<br>SCE/BP/4929-WO | Date of mailing *(day/month/year)*<br>26/03/2001 |
| International application No.<br>PCT/GB 00/ 03301 | **PAYMENT DUE** within 30 ~~months~~/days from the above date of mailing |
| Applicant<br>NYCOMED AMERSHAM PLC... | International filing date *(day/month/year)*<br>30/08/2000 |

1. This International Searching Authority

(i) considers that there are 8 *(number of)* inventions claimed in the international application covered by the claims indicated ~~below~~/on the extra sheet:

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated ~~below~~/on the extra sheet:

(ii) [X] has carried out a partial international search (see Annex) [ ] will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:

1-10(partially),12-15(partially)

(iii) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid

2. The applicant is hereby **invited,** within the time limit indicated above, to pay the amount indicated below:

GBP 624,00 x 7 = GBP 4.368,00
Fee per additional invention | number of additional inventions | total amount of additional fees

Or, EUR 945,00 x 7 = EUR 6.615,00

The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fee may be made under protest,** i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive.

3. [ ] Claim(s) Nos. _____ have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

| | |
|---|---|
| Name and mailing address of the International Searching Authority<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Authorized officer<br>John De Bruijn |

Form PCT/ISA/206 (July 1992)

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-10 (partially), 12-15 (partially)

   A compound of the general chemical formula of claim 1 wherein Q is a sugar and X=CH; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

2. Claims: 1-10 (partially), 12-15 (partially)

   A compound of the general chemical formula of claim 1 wherein Q is a sugar and X=N; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

3. Claims: 1-10 (partially), 12-15 (partially)

   A compound of the general chemical formula of claim 1 wherein Q is a sugar analogue and X=CH, provided that Q is not a sugar; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

4. Claims: 1-10 (partially), 12-15 (partially)

   A compound of the general chemical formula of claim 1 wherein Q is a sugar analogue and X=N, provided that Q is not a sugar; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

5. Claims: 1-15 (partially)

   A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone and X=CH, provided that Q is neither a sugar nor a sugar analogue; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

6. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone and X=N, provided that Q is neither a sugar nor a sugar analogue; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

7. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone analogue and X=CH, provided that Q is neither a sugar nor a sugar analogue nor a nucleic acid backbone; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

8. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone analogue and X=N, provided that Q is neither a sugar nor a sugar analogue nor a nucleic acid backbone; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

The problem to be solved by the present application is to provide further labelled imidazolinone analogues for incorporation into oligonucleotides or analogues thereof and useful in a wide variety of research and diagnostic techniques. The solution is the provision of the compounds described by the Markush formula of claim 1.
The single general inventive concept underlying the claimed inventions is the teaching that labelled compounds represented by the above-mentioned formula can be incorporated into oligonucleotide analogues for use in well-known research and diagnosic techniques.
Document D1 (JP61171497, see WPI abstract) and document D2 (Z. Naturforsch. (1986) 41b, 1571-1579, see page 1571 to page 1572 first paragraph and compound 1) both disclose imidazolinones used in probe cDNA oligomers and comprising 1-(2-deoxy-beta-D-ribofuranosyl)-2-oxo-4-imidazoline-4-carboxamides the chemical structure of which falls within the scope of claim 1. Document D3 (WO94/21658, see page 7 line 10 to page 10 line 16, page 21 line 21 to page 22 line 2 and scheme page 60) discloses imidazolinone nucleoside derivatives which can be incorporated into DNA while remaining accessible to enzymes.

These documents destroy the novelty of the afore-mentioned single general concept.

Therefore, no single general inventive concept underlying a unique invention (or a group of inventions) can be found in the present application and the technical features of the above-mentioned solution cannot be regarded as special technical features in the sense of Rule 13.2 PCT. According to Rules 13.1 and 13.2 PCT, there is lack of unity and the different inventions not belonging to a common inventive concept (in light of the prior art) are formulated as the different subjects on the communication pursuant to Article 17(3)(a) PCT.

Remarks

1) The number of subjects has been kept as low as possible with due regard to the teaching of T110/82 (OJ EPO 1983 p. 274-281) concerning the balance between:

- a rational procedure up to grant in which the interconnected matter should no needlesly be split up nor unrelated invenions lumped together for the purpose of saving fees;

- and an equitable levying of fees, especially in respect of search, since the expense must be borne by the fees levied for other applications.

2) Detecting documents necessary for establishing whether the requirements of the PCT relating to novelty and inventive step of each of the inventions would have required major additional searching efforts.

3) The search has been carried out only with respect to the first subject. It therefore does not imply acknowledgement of unity of invention within the further identified subjects i.e. within each of the groups of inventions mutually linked by hitherto unsearched common concepts. These concepts might, in the course of additional searches, if any, reveal themselves as lacking novelty or inventive step and hence uncover a further lack of unity.

Annex to Form PCT/ISA/206

COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
PCT/GB 00/03301

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
see 'Invitation to pay additional fees'
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.

3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.

4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | FUKUDA T. ET AL.: "An alternative to the mixed probe method in DNA hybridization: synthetic "lure" nucleotide for the ambiguous position of codons" Z. NATURFORSCH., vol. 41b, 1986, pages 1571-1579, XP002161692 cited in the application page 1571 -page 1572, left-hand column, paragraph 1 * page 1572, compounds 1, 3-6 * page 1572, right-hand column, last paragraph -page 1573, left-hand column, paragraph 1 * page 1573, compounds 14 and 15 * --- | 1-3,5-10 |
| X | DATABASE WPI Section Ch, Week 198637 Derwent Publications Ltd., London, GB; Class B03, AN 1986-242387 XP002131974 -& JP 61 171497 A (TAKEDA CHEM IND LTD), 2 August 1986 (1986-08-02) figure 3 RN 105386-25-2, 105386-26-3 and for instance 105386-27-4 abstract --- -/-- | 1-10 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Form PCT/ISA/206 (Annex, first sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | BEDU E ET AL: "Novel 2'-Deoxycytidine Analogues as pH Independent Substitutes of Protonated Cytosines in Triple Helix Forming Oligonucleotides" TETRAHEDRON LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 40, no. 5, 29 January 1999 (1999-01-29), pages 835-838, XP004151459 ISSN: 0040-4039 cited in the application page 835 scheme 3 --- | 1-3,5-10 |
| X | WO 98 16186 A (ICN PHARMACEUTICALS ;AVERETT DEVRON (US); TAM ROBERT (US); RAMASAM) 23 April 1998 (1998-04-23) figure 9 --- | 1-3,5-8 |
| X | WO 94 21658 A (KALMAN THOMAS I) 29 September 1994 (1994-09-29) cited in the application page 12 -page 13 claims --- | 1-3,5-9 |
| X | DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; JIANG, XIANG-JUN ET AL: "Synthesis of a novel antiretroviral thymidine analog: 1-(2-deoxy-.beta.-D-ribofuranosyl)-4-acety limidazolin-2-one (imidine)" retrieved from STN Database accession no. 121:83843 XP002161694 abstract RN 156357-03-8, 156357-04-9, 156357-05-0 and 156357-06-1 & NUCLEOSIDES NUCLEOTIDES (1994), 13(1-3), 379-88 , --- | 1-3,5-8 |
| X | KALMAN, T. I. ET AL: "Mechanism of inhibition of HIV reverse transcriptase by 1-(2-deoxy-.beta.-D-ribofuranosyl)-4-acety limidazolin-2-one (imidine)" NUCLEOSIDES NUCLEOTIDES (APRIL-MAY 1999), 18(4 & 5), 847-848, XP002161693 RN 156357-05-0 the whole document --- -/-- | 1-3,5-12 |

2

International Application No
PCT/GB 00/03301

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | SCHMIDT R -M ET AL: "BIOSYNTHESIS OF 4-FORMYL-4-IMIDAYOLINE-2-ON THE HETEROCYCLIC BASE OF NIKKOMYCIN X" ZEITSCHRIFT FUER NATURFORSCHUNG. SECTION C. BIOSCIENCES,XX,XX, vol. 41, no. 1/02, 1986, pages 135-140, XP000884825 ISSN: 0341-0382 page 135 page 139, left-hand column, last paragraph --- | 1,3,5-8 |
| X | HAGENMAIER, HANSPAUL ET AL: "Metabolites of microorganisms. 182. Structure elucidation of the nucleoside antibiotic nikkomycin X" LIEBIGS ANN. CHEM. (1979), (10), 1494-502, XP002131972 page 1494 -page 1495 * page 1499, compound 5 * ----- | 1,3,5-8 |

# Patent Family Annex

Information on patent family members

International Application No
PCT/GB 00/03301

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| JP 61171497 A | 02-08-1986 | NONE | |
| WO 9816186 A | 23-04-1998 | AU 4986797 A | 11-05-1998 |
| | | BR 9712527 A | 08-03-2000 |
| | | CN 1268140 A | 27-09-2000 |
| | | CZ 9901206 A | 15-09-1999 |
| | | EP 1027359 A | 16-08-2000 |
| | | NO 991785 A | 15-06-1999 |
| | | PL 333419 A | 06-12-1999 |
| | | SI 20076 A | 30-04-2000 |
| | | US 6130326 A | 10-10-2000 |
| WO 9421658 A | 29-09-1994 | NONE | |

# PATENT COOPERATION TREATY

RECEIVED
- 7 FEB 2002
CH/AJR/LC/MH

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

HAMMER, ROLLINS, CANNING & HAMMETT
Nycomed Amersham
White Lion Road
Amersham
Buckinghamshire, HP7 9LL
GRANDE BRETAGNE

# PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

fax Confirmation

| | |
|---|---|
| Date of mailing *(day/month/year)* | 04.02.2002 |
| Applicant's or agent's file reference: PA9943-PCT | **IMPORTANT NOTIFICATION** |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/GB00/03301 | 30/08/2000 | 31/08/1999 |

Applicant
NYCOMED AMERSHAM PLC...

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

| | |
|---|---|
| DUE DATE: | N/A |
| FORMALITIES: | JHV |
| PAT. OFF: | IPH |
| ~~ON DB:~~ | ~~7/12/02~~ |
| CASE NO: | PA9943 |

Name and mailing address of the IPEA/

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized officer

Cardenas, C

Tel.+31 70 340-3370

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| PA9943-PCT | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/GB00/03301 | 30/08/2000 | 31/08/1999 |
| International Patent Classification (IPC) or national classification and IPC<br>C07H19/00 | | |
| Applicant<br>NYCOMED AMERSHAM PLC... | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 26/03/2001 | 04.02.2002 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office - P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk - Pays Bas<br>Tel. +31 70 340 - 2040 Tx: 31 651 epo nl<br>Fax: +31 70 340 - 3016 | Authorized officer<br>Held, P<br>Telephone No. +31 70 340 2830 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03301

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
**Description, pages:**

1-57 as originally filed

**Claims, No.:**

1-15 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-10, 12-15 (all partly), 11.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-10, 12-15 (all partly), 11.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 13-15 (all partly) |
| | No: | Claims | 1-10,12 (all partly) |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-10,12-15 (all partly) |
| Industrial applicability (IA) | Yes: | Claims | 1-10, 12-15 (all partly) |

No: Claims

2. Citations and explanations
**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
**see separate sheet**

Re Item III

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

Claims 1-10 and 12-15 were examined in as far as they relate to a compound of the general chemical formula of claim 1 wherein Q is a sugar and X=CH; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

**NOVELTY**

Documents D1-D9 disclose nucleosides/nucleotides encompassed by the Markush formulae of claim 1 and uses thereof. See for example in D1 (page 998, scheme 3) compound (f) (X=CH, Y=CO, W=Rp=$NH_2$) or (d) (X=CH, Y=CO, W=Ln-Rp, Ln=O, Rp=$N(C(O)CH_2)_2$).

Therefore, **claims 1-10 and 12** are not novel in the sense of Article 33 (2) PCT.

**INVENTIVE STEP**

The problem to be solved by the present application is to provide further compounds for incorporation into oligonucleotides or analogues thereof and useful in a wide variety of research and diagnostic techniques. The solution is the provision of compounds described by the Markush formulae of claim 1.

Document D1 (see WPI abstract) and document D2 (see page 1571 to page 1572 first paragraph and compound 1) both disclose imidazolinones used in probe cDNA oligomers and comprising 1-(2-deoxy-beta-D-ribofuranosyl)-2-oxo-4-imidazoline- 4-carboxamides the chemical structure of which falls within the scope of claim 1.

Therefore, **claims 1-10, 12-15** are not inventive in the sense of Article 33 (3) PCT.

**Re It m VIII**

Certain observations on the international application.

The terms "reporter moiety" and "sugar" used in claims 1-10, 12-15 are vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

ATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference | FOR FURTHER ACTION | see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. |
|---|---|---|
| SCE/BP/4929-WO | | |
| International application No. | International filing date *(day/month/year)* | (Earliest) Priority Date *(day/month/year)* |
| PCT/GB 00/ 03301 | 30/08/2000 | 31/08/1999 |
| Applicant | | |
| NYCOMED AMERSHAM PLC... | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readble form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. -

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/03301

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10 (partially), 12-15 (partially)

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-10 (partially), 12-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a sugar and X=CH; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

2. Claims: 1-10 (partially), 12-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a sugar and X=N; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

3. Claims: 1-10 (partially), 12-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a sugar analogue and X=CH, provided that Q is not a sugar; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

4. Claims: 1-10 (partially), 12-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a sugar analogue and X=N, provided that Q is not a sugar; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

5. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone and X=CH, provided that Q is neither a sugar nor a sugar analogue; a nucleoside/nucleotide analogue comprising such a compound; a

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

6. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone and X=N, provided that Q is neither a sugar nor a sugar analogue; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

7. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone analogue and X=CH, provided that Q is neither a sugar nor a sugar analogue nor a nucleic acid backbone; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

8. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone analogue and X=N, provided that Q is neither a sugar nor a sugar analogue nor a nucleic acid backbone; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/03301

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H19/052 C07H21/00 C07D249/12 C07D249/14 C07D233/66

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | FUKUDA T. ET AL.: "An alternative to the mixed probe method in DNA hybridization: synthetic "lure" nucleotide for the ambiguous position of codons" Z. NATURFORSCH., vol. 41b, 1986, pages 1571-1579, XP002161692 cited in the application page 1571 -page 1572, left-hand column, paragraph 1 * page 1572, compounds 1, 3-6 * page 1572, right-hand column, last paragraph -page 1573, left-hand column, paragraph 1 * page 1573, compounds 14 and 15 * --- -/-- | 1-3,5-10 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 28 February 2001 | 17. 05. 2001 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Held, P |

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | DATABASE WPI<br>Section Ch, Week 198637<br>Derwent Publications Ltd., London, GB;<br>Class B03, AN 1986-242387<br>XP002131974<br>-& JP 61 171497 A (TAKEDA CHEM IND LTD),<br>2 August 1986 (1986-08-02)<br>figure 3<br>RN 105386-25-2, 105386-26-3 and for<br>instance 105386-27-4<br>abstract | 1-10 |
| | --- | |
| X | BEDU E ET AL: "Novel 2'-Deoxycytidine<br>Analogues as pH Independent Substitutes of<br>Protonated Cytosines in Triple Helix<br>Forming Oligonucleotides"<br>TETRAHEDRON LETTERS,NL,ELSEVIER SCIENCE<br>PUBLISHERS, AMSTERDAM,<br>vol. 40, no. 5,<br>29 January 1999 (1999-01-29), pages<br>835-838, XP004151459<br>ISSN: 0040-4039<br>cited in the application<br>page 835<br>scheme 3 | 1-3,5-10 |
| | --- | |
| X | WO 98 16186 A (ICN PHARMACEUTICALS<br>;AVERETT DEVRON (US); TAM ROBERT (US);<br>RAMASAM) 23 April 1998 (1998-04-23)<br>figure 9 | 1-3,5-8 |
| | --- | |
| X | WO 94 21658 A (KALMAN THOMAS I)<br>29 September 1994 (1994-09-29)<br>cited in the application<br>page 12 -page 13<br>claims | 1-3,5-9 |
| | --- | |
| X | DATABASE CHEMABS [Online]<br>CHEMICAL ABSTRACTS SERVICE, COLUMBUS,<br>OHIO, US;<br>JIANG, XIANG-JUN ET AL: "Synthesis of a<br>novel antiretroviral thymidine analog:<br>1-(2-deoxy-.beta.-D-ribofuranosyl)-4-acety<br>limidazolin-2-one (imidine)"<br>retrieved from STN<br>Database accession no. 121:83843<br>XP002161694<br>abstract<br>RN 156357-03-8, 156357-04-9, 156357-05-0<br>and 156357-06-1<br>& NUCLEOSIDES NUCLEOTIDES (1994), 13(1-3),<br>379-88, | 1-3,5-8 |
| | --- | |
| | -/-- | |

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | KALMAN, T. I. ET AL: "Mechanism of inhibition of HIV reverse transcriptase by 1-(2-deoxy-.beta.-D-ribofuranosyl)-4-acety limidazolin-2-one (imidine)" NUCLEOSIDES NUCLEOTIDES (APRIL-MAY 1999), 18(4 & 5), 847-848, XP002161693 RN 156357-05-0 the whole document --- | 1-3,5-12 |
| X | SCHMIDT R -M ET AL: "BIOSYNTHESIS OF 4-FORMYL-4-IMIDAYOLINE-2-ON THE HETEROCYCLIC BASE OF NIKKOMYCIN X" ZEITSCHRIFT FUER NATURFORSCHUNG. SECTION C. BIOSCIENCES,XX,XX, vol. 41, no. 1/02, 1986, pages 135-140, XP000884825 ISSN: 0341-0382 page 135 page 139, left-hand column, last paragraph --- | 1,3,5-8 |
| X | HAGENMAIER, HANSPAUL ET AL: "Metabolites of microorganisms. 182. Structure elucidation of the nucleoside antibiotic nikkomycin X" LIEBIGS ANN. CHEM. (1979), (10), 1494-502, XP002131972 page 1494 -page 1495 * page 1499, compound 5 * ----- | 1,3,5-8 |

2

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/03301

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| JP 61171497 A | 02-08-1986 | NONE | |
| WO 9816186 A | 23-04-1998 | AU 4986797 A | 11-05-1998 |
| | | BR 9712527 A | 08-03-2000 |
| | | CN 1268140 A | 27-09-2000 |
| | | CZ 9901206 A | 15-09-1999 |
| | | EP 1027359 A | 16-08-2000 |
| | | NO 991785 A | 15-06-1999 |
| | | PL 333419 A | 06-12-1999 |
| | | SI 20076 A | 30-04-2000 |
| | | US 6130326 A | 10-10-2000 |
| WO 9421658 A | 29-09-1994 | NONE | |

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/16150 A3

(51) International Patent Classification[7]: C07H 19/052, 21/00, C07D 249/12, 249/14, 233/66

(21) International Application Number: PCT/GB00/03301

(22) International Filing Date: 30 August 2000 (30.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
99306887.3 31 August 1999 (31.08.1999) EP

(71) Applicant *(for all designated States except US)*: **NYCOMED AMERSHAM PLC** [GB/GB]; Amersham Laboratories, White Lion Road, Amersham, Buckinghamshire HP7 9LL (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SMITH, Clifford** [GB/GB]; 27 Hunters Close, Tring, Herts HP23 5PG (GB). **CUMMINS, William, Jonathan** [GB/GB]; 5 Thorntree Drive, Tring, Herts HP23 4JE (GB). **NAIRNE, Robert, James, Domett** [GB/GB]; 16 Springwood Walk, St Albans, Herts AL4 9UN (GB).

(74) Agent: **EASTWOOD, Simon, Christopher**; Stevens Hewlett & Perkins, 1 St Augustine's Place, Bristol BS1 4UD (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

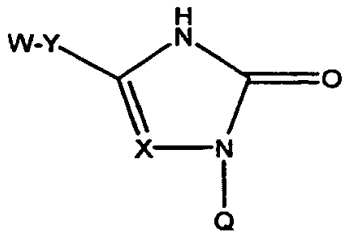
**Published:**
— *with international search report*

(88) Date of publication of the international search report:
15 November 2001

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/16150 A3

(54) Title: NUCLEOSIDE ANALOGUES




(I)

(57) Abstract: Compounds having structure (I) where X is CH or N, Y is -CO-, -CONW-, -O-, -S-, -SO-, -$SO_2$-, -NWCO-, -NW-, or -OCO-, W is the same or different at different places in the molecule and each is H or alkyl or aryl or Rp or -Ln-Rp, Ln is a linker group, Rp is a reporter moiety, and Q is a sugar or a sugar analogue or a nucleic acid backbone or backbone analogue, provided that at least one reporter moiety Rp is present, provide nucleoside triphosphates which are good enzyme substrates.

# INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/GB 00/03301

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H19/052 C07H21/00 C07D249/12 C07D249/14 C07D233/66

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | FUKUDA T. ET AL.: "An alternative to the mixed probe method in DNA hybridization: synthetic "lure" nucleotide for the ambiguous position of codons" Z. NATURFORSCH., vol. 41b, 1986, pages 1571-1579, XP002161692 cited in the application page 1571 -page 1572, left-hand column, paragraph 1 * page 1572, compounds 1, 3-6 * page 1572, right-hand column, last paragraph -page 1573, left-hand column, paragraph 1 * page 1573, compounds 14 and 15 * --- -/-- | 1-3,5-10 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 28 February 2001 | 17. 05. 2001 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Held, P |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/GB 00/03301

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | DATABASE WPI<br>Section Ch, Week 198637<br>Derwent Publications Ltd., London, GB;<br>Class B03, AN 1986-242387<br>XP002131974<br>-& JP 61 171497 A (TAKEDA CHEM IND LTD),<br>2 August 1986 (1986-08-02)<br>figure 3<br>RN 105386-25-2, 105386-26-3 and for<br>instance 105386-27-4<br>abstract | 1-10 |
| | --- | |
| X | BEDU E ET AL: "Novel 2'-Deoxycytidine<br>Analogues as pH Independent Substitutes of<br>Protonated Cytosines in Triple Helix<br>Forming Oligonucleotides"<br>TETRAHEDRON LETTERS,NL,ELSEVIER SCIENCE<br>PUBLISHERS, AMSTERDAM,<br>vol. 40, no. 5,<br>29 January 1999 (1999-01-29), pages<br>835-838, XP004151459<br>ISSN: 0040-4039<br>cited in the application<br>page 835<br>scheme 3 | 1-3,5-10 |
| | --- | |
| X | WO 98 16186 A (ICN PHARMACEUTICALS<br>;AVERETT DEVRON (US); TAM ROBERT (US);<br>RAMASAM) 23 April 1998 (1998-04-23)<br>figure 9 | 1-3,5-8 |
| | --- | |
| X | WO 94 21658 A (KALMAN THOMAS I)<br>29 September 1994 (1994-09-29)<br>cited in the application<br>page 12 -page 13<br>claims | 1-3,5-9 |
| | --- | |
| X | DATABASE CHEMABS [Online]<br>CHEMICAL ABSTRACTS SERVICE, COLUMBUS,<br>OHIO, US;<br>JIANG, XIANG-JUN ET AL: "Synthesis of a<br>novel antiretroviral thymidine analog:<br>1-(2-deoxy-.beta.-D-ribofuranosyl)-4-acety<br>limidazolin-2-one (imidine)"<br>retrieved from STN<br>Database accession no. 121:83843<br>XP002161694<br>abstract<br>RN 156357-03-8, 156357-04-9, 156357-05-0<br>and 156357-06-1<br>& NUCLEOSIDES NUCLEOTIDES (1994), 13(1-3),<br>379-88, | 1-3,5-8 |
| | --- | |
| | -/-- | |

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/GB 00/03301

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | KALMAN, T. I. ET AL: "Mechanism of inhibition of HIV reverse transcriptase by 1-(2-deoxy-.beta.-D-ribofuranosyl)-4-acety limidazolin-2-one (imidine)" NUCLEOSIDES NUCLEOTIDES (APRIL-MAY 1999), 18(4 & 5), 847-848, XP002161693 RN 156357-05-0 the whole document --- | 1-3,5-12 |
| X | SCHMIDT R -M ET AL: "BIOSYNTHESIS OF 4-FORMYL-4-IMIDAYOLINE-2-ON THE HETEROCYCLIC BASE OF NIKKOMYCIN X" ZEITSCHRIFT FUER NATURFORSCHUNG. SECTION C. BIOSCIENCES,XX,XX, vol. 41, no. 1/02, 1986, pages 135-140, XP000884825 ISSN: 0341-0382 page 135 page 139, left-hand column, last paragraph --- | 1,3,5-8 |
| X | HAGENMAIER, HANSPAUL ET AL: "Metabolites of microorganisms. 182. Structure elucidation of the nucleoside antibiotic nikkomycin X" LIEBIGS ANN. CHEM. (1979), (10), 1494-502, XP002131972 page 1494 -page 1495 * page 1499, compound 5 * ----- | 1,3,5-8 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/GB 00/03301

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10 (partially), 12-15 (partially)

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-10 (partially), 12-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a sugar and X=CH; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

2. Claims: 1-10 (partially), 12-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a sugar and X=N; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

3. Claims: 1-10 (partially), 12-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a sugar analogue and X=CH, provided that Q is not a sugar; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

4. Claims: 1-10 (partially), 12-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a sugar analogue and X=N, provided that Q is not a sugar; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

5. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone and X=CH, provided that Q is neither a sugar nor a sugar analogue; a nucleoside/nucleotide analogue comprising such a compound; a

polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

6. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone and X=N, provided that Q is neither a sugar nor a sugar analogue; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

7. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone analogue and X=CH, provided that Q is neither a sugar nor a sugar analogue nor a nucleic acid backbone; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

8. Claims: 1-15 (partially)

A compound of the general chemical formula of claim 1 wherein Q is a nucleic acid backbone analogue and X=N, provided that Q is neither a sugar nor a sugar analogue nor a nucleic acid backbone; a nucleoside/nucleotide analogue comprising such a compound; a polynucleotide chain comprising such a nucleoside analogue; a chain extension method which comprises reacting such a polynucleotide chain and a method of detecting a nucleic acid which contains the aforementioned compound.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Int onal Application No
PCT/GB 00/03301

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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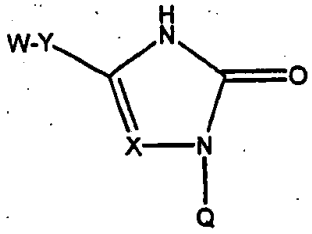
(54) Title: NUCLEOSIDE ANALOGUES




(I)

(57) Abstract: Compounds having structure (I) where X is CH or N, Y is -CO-, -CONW-, -O-, -S-, -SO-, -$SO_2$-, -NWCO-, -NW-, or -OCO-, W is the same or different at different places in the molecule and each is H or alkyl or aryl or Rp or -Ln-Rp, Ln is a linker group, Rp is a reporter moiety, and Q is a sugar or a sugar analogue or a nucleic acid backbone or backbone analogue, provided that at least one reporter moiety Rp is present, provide nucleoside triphosphates which are good enzyme substrates.

WO 01/16150 A2

# NUCLEOSIDE ANALOGUES

## Introduction

The present invention relates to compounds suitable for use as nucleoside analogues, and to polynucleotide chains comprising nucleoside analogues.

Nucleic acids are manipulated *in vitro* in a wide variety of research and diagnostic techniques. The methods can involve the synthesis of nucleic acid probes by means of DNA or RNA polymerase, reverse transcriptase or terminal transferase enzymes for the purposes of labelling or determination of base sequence identity. Labelling often involves the incorporation of a nucleotide which is chemically labelled or which is of a particular chemical composition so as to make it detectable. Nucleic acid probes made in this way can be used to determine the presence of a nucleic acid target which has a complementary sequence by means of hybridisation of the probe to the target.

In WO 94/21658 T I Kalman describes novel nucleoside or nucleotide analogues having a 4-acetylimidazolin-2-one base and their use for inhibiting virally encoded reverse transcriptases.

In Z Naturforsch B, 1986, 41b (12), 1571-9, T Fukuda *et al* describe the effect of incorporation of nucleoside analogues having an imidazolin-2-one base as both T and G in DNA duplexes.

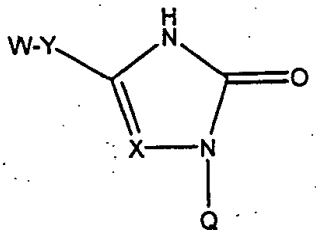
In Tetrahedron Letters, 40 (1999), 835-838, E Bedu *et al* describe the preparation of a nucleoside analogue having a 4-amidoimidazolin-2-one base and used as a cytosine analogue in triple helix forming oligonucleotides.

Purine and pyrimidine base nucleosides and nucleotides have been derivatised with reporter groups and are well known and widely used

for lab lling DNA or RNA and in other molecular biology applications. But these molecules are often poor enzyme substrates. There is a continuing need for labelled nucleoside analogues whose triphosphates are good enzyme substrates.

**Statement of Invention**

According to the present invention there is provided a compound having the structure




where X is CH or N,

Y is -CO-, -CONW-, -O-, -S-, -SO-, -$SO_2$-, -NWCO-,-NW-, or -OCO-,

W is the same or different at different places in the molecule and each is H or alkyl or aryl or Rp or –Ln–Rp,

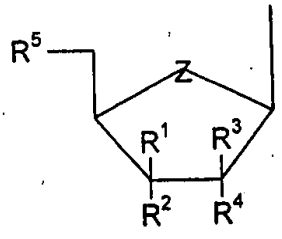
Ln is a linker group,

Rp is a reporter moiety, and

Q is a sugar or a sugar analogue or a nucleic acid backbone or backbone analogue,

provided that at least one reporter moiety Rp is present.

Q may be




where Z is O, S, Se, SO, NW or $CH_2$,

$R^1$, $R^2$, $R^3$ and $R^4$ are the same or different and each is H,

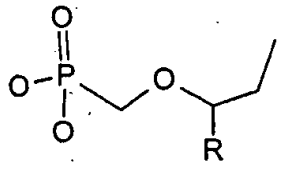
OH, F, $NH_2$, $N_3$, O-hydrocarbyl or Rp or –Ln–Rp,

$R^5$ is OH, SH or $NH_2$ or mono-, di- or tri-phosphate or -thiophosphate, or corresponding boranophosphate,

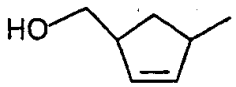
or one of $R^2$ and $R^5$ is a phosphoramidite or other group for incorporation in a polynucleotide chain, or Rp or –Ln–Rp,

or Q consists of one of the following modified sugar structures

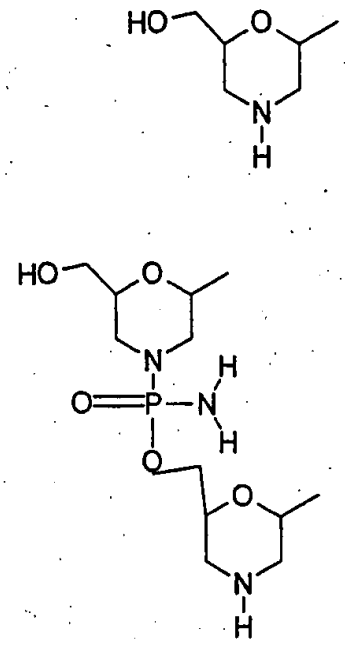
Acyclic Sugars



R= $CH_3$ , $CH_2OH$ , H ,



Morpholino Backbone



or Q is a nucleic acid backbone consisting of sugar-phosphate repeats or modified sugar-phosphate repeats (e.g. LNA) (Koshkin *et al*, 1998, Tetrahedron 54, 3607-30) or a backbone analogue such as peptide or polyamide nucleic acid (PNA). (Nielsen *et al*, 1991, Science 254, 1497 - 1500).

The reporter moiety Rp, with or without the linker group Ln, will be present in W or Y when Y contains W and will always have at least one atom between the reporter and the base ring. When Q is a sugar or sugar analogue or a modified sugar, these compounds are nucleotide analogues or nucleoside analogues. When Q is a nucleic acid backbone or a backbone analogue, these compounds are herein called nucleic acids or polynucleotides.

A nucleoside analogue is a molecule which is capable of being incorporated, either chemically or enzymatically, into an oligomeric or polymeric nucleic acid (DNA or RNA) chain, and when so incorporated of

forming a base pair with a nucleotide residue in a complementary chain or base stacking in the appropriate nucleic acid chain.

In the context of this invention, a nucleotide is a naturally occurring compound comprising a heterocyclic base and a sugar moiety including a phosphate. A nucleoside is a corresponding compound in which a phosphate is not present. Nucleotide analogues and nucleoside analogues are analogous compounds having different bases and/or different sugar moieties. A nucleoside analogue is a compound which is capable of forming part of a nucleic acid (DNA or RNA or PNA) chain, and is there capable of base-pairing with a base in a complementary chain or base stacking in the appropriate nucleic acid chain. A nucleoside analogue may be specific, by pairing with only one complementary nucleotide; or degenerate, by base pairing with more than one of the natural bases, e.g. with pyrimidines (T/C) or purines (A/G); or universal, by pairing with each of the natural bases with little discrimination; or it may pair with another analogue or itself.

In one preferred aspect of the invention, the base analogue is linked to a sugar moiety such as ribose, deoxyribose or dideoxyribose to form a nucleoside analogue. When the group $R^5$ is triphosphate, the nucleoside triphosphate analogues of the invention are capable of being incorporated by enzymatic means into nucleic acid chains.

A reporter moiety Rp may be any one of various things. It may be a radioisotope by means of which the nucleoside analogue is rendered easily detectable, for example 32-P or 33-P or 35-S incorporated in a phosphate or thiophosphate or phosphoramidite or H-phosphonate group, or alternatively 3-H or 14-C or 125-I. It may be a stable isotope or a specific chemical moiety suitable for detection by mass spectrometry. (Or the compound as a whole may be suitable for detection by mass spectrometry.) It may be a signal moiety e.g. an enzyme, hapten, fluorophore, chemiluminescent group, Raman label or electrochemical label.

The reporter moiety may be a solid surface, to which the nucleoside analogue is attached and by means of which it may be distinguished from nucleoside analogues not so immobilised. The reporter moiety may be a reactive group, either a nucleophilic group, e.g. $NH_2$, OH, COOH, $CONH_2$, $ONH_2$, SH or a thiophosphate or a hydrazine or a hydrazide, or an electrophilic group e.g. an active ester or aldehyde or maleimide, by which a signal moiety and/or a solid surface may be attached, before or after incorporation of the nucleoside analogue in a nucleic acid chain. Such reporter groups are well known and well described in the literature.

A linker group Ln is a chain of 1 to 60 or more carbon, nitrogen, oxygen phosphorus and/or sulphur atoms, rigid or flexible, saturated or unsaturated, as well known in the field. Preferably the linker group is joined to a 4-triazole ring (when X is N) or to a 4-imidazole ring (when X is CH) of the nucleoside analogue molecule by a group having an alpha carbonyl group, e.g. amide or an amine bond. Preferably the linker group is joined to the reporter moiety by an amide bond.

To avoid risk of steric hindrance, a linker preferably has at least three chain atoms, e.g. $-(CH_2)_n-$ where n is at least 3.

Two (or more) reporter moieties may be present, e.g. a signal moiety and a solid surface, or a hapten and a different signal moiety, or two fluorescent signal groups to act as donor and acceptor. Various formats of these arrangements may be useful for separation or detection purposes.

Purine and pyrimidine nucleoside derivatives labelled with reporter moieties are well known and well described in the literature. Labelled nucleoside derivatives have the advantage of being readily detectable during sequencing or other molecular biology techniques.

$R^1$, $R^2$, $R^3$ and $R^4$ may each be H, OH, F, $NH_2$, $N_3$, O-alkyl or a reporter moiety. Thus ribonucleosides, and deoxyribonucleosides and dideoxyribonucleosides are envisaged together with other nucleoside analogues. These sugar substituents may contain a reporter moiety in

place of or in addition to the one or two present in the base.

$R^5$ is OH or mono-, di- or tri-phosphate or -thiophosphate or corresponding boranophosphate. From nucleosides ($R^5$ is OH) it is readily possible to make the corresponding nucleotides ($R^5$ is triphosphate) by literature methods. Alternatively, one of $R^2$ and $R^5$ may be a phosphoramidite or H-phosphonate or methylphosphonate or phosphorothioate or amide, or an appropriate linkage to a solid surface e.g. hemisuccinate controlled pore glass, or other group for incorporation , generally by chemical means, in a polynucleotide chain. The use of phosphoramidites and related derivatives in synthesising oligonucleotides is well known and described in the literature.

In the new nucleoside analogues to which this invention is directed, at least one reporter moiety is present preferably in the base analogue and/or optionally in the sugar moiety or a phosphate group. Reporter moieties may be introduced into the sugar moiety of a nucleosid analogue by literature methods (e.g. J. Chem. Soc. Chem. Commun. 1990, 1547-8; J. Med. Chem., 1988, 31. 2040-8). Reporter moieties in the form of isotopic labels may be introduced into phosphate groups by literature methods (Analytical Biochemistry, 214, 338-340, 1993; WO 95/15395).

When $R^5$ is triphosphate, the nucleoside analogues are available for enzymatic incorporation in DNA or RNA. The invention includes in another aspect the polynucleotide chain comprising at least one residue of the nucleoside analogue as defined.

Nucleoside analogues of this invention are useful for labelling DNA or RNA or for incorporating in oligonucleotides or PNA. A reporter moiety is attached at a position where it does not have a significant detrimental effect on the physical or biochemical properties of the nucleoside analogue, in particular its ability to be incorporated in single stranded or double stranded nucleic acid.

A template containing the incorporated nucleoside analogue of this invention may be suitable for copying in nucleic acid synthesis. If a

reporter moiety of the incorporated nucleoside analogue consists of a linker group, then a signal moiety can be introduced into the incorporated nucleoside analogue by being attached through a terminal or other reactive group of the linker group.

A nucleoside analogue triphosphate of this invention may be incorporated by enzymes such as terminal transferase to extend the 3' end of nucleic acid chains in a non-template directed manner. Tails of the nucleoside analogue triphosphate produced in this way may be detected directly in the absence of any reporter label by use of antibodies directed against the nucleoside analogue. The analogues when incorporated into oligonucleotides or nucleic acids may be acted upon by nucleic acid modification enzymes such as ligases or restriction endonucleases.

The nucleoside analogues of this invention can also be used in any of the existing applications which use native nucleic acid probes labelled with haptens, fluorophores or other reporter groups, for example on Southern blots, dot blots and in polyacrylamide or agarose gel based methods or solution hybridisation assays and other assays in microtitre plates or tubes or assays of oligonucleotides or nucleic acids such as on microchips. The probes may be detected with antibodies targeted either against haptens which are attached to the base analogues or against the base analogues themselves which would be advantageous in avoiding additional chemical modification. Antibodies used in this way are normally labelled with a detectable group such as a fluorophore or an enzyme. Fluorescent detection may also be used if the base analogue itself is fluorescent or if there is a fluorophore attached to the nucleoside analogue.

RNA is an extremely versatile biological molecule. Experimental studies by several laboratories have shown that in vitro selection techniques can be employed to isolate short RNA molecules from RNA libraries that bind to proteins, not normally associated with RNA binding, including a few antibodies, with high affinity and specificity (Gold, Allen, Binkley, et al,1993, 497-510 in The RNA World, Cold Spring Harbor

Press, Cold Spring Harbor N.Y., Gold, Polisky, Unlenbeck, and Yarus, 1995, Annu. Rev. Biochem. 64: 763-795, Tuerk and Gold, 1990, Science 249:505-510, Joyce, 1989, Gene 82:83-87, Szostak, 1992, Trends Biochem. Sci 17:89-93, Tsai, Kenan and Keene, 1992, PNAS 89:8864-8868, Tsai, Kenan and Keene, 1992, PNAS 89:8864-8868, Doudna, Cech and Sullenger, 1995, PNAS 92:2355-2359). Some of these RNA molecules have been proposed as drug candidates for the treatment of diseases like myasthenia gravis and several other auto-immune diseases.

The basic principle involves adding an RNA library to the protein or molecule of interest. Washing to remove unbound RNA. Then specifically eluting the RNA bound to the protein. The RNA is then reverse transcribed and amplified by PCR. The DNA is then transcribed using modified nucleotides (either 2' modifications to give nuclease resistance e.g. 2' F, 2' $NH_2$, 2' $OCH_3$ and/or C5 modified pyrimidines and/or C8 modified purines). Those molecules that are found to bind the protein or other molecule of interest are cloned and sequenced to look for common sequences. The common sequence is taken and used to make a short oligonucleotide therapeutic.

The base analogues described here, when converted to the nucleoside triphosphate or nucleoside phosphoramidite, will significantly increase the molecular diversity available for this selection process. This may lead to oligonucleotides with increased binding affinity to the target that is not available from the current building blocks.

The use of triphosphate nucleotide analogues containing five membered heterocycles such as pyrrole have been demonstrated to act as substrates for enzymatic incorporation, (WO 97/28176). The nucleotide base analogue pyrrole-3,4-dicarboxamide is a particularly good substrate. The corresponding base analogue pyrrole-3-carboxamide is also a substrate but with a significant decrease in efficiency relative to the dicarboxamide. This illustrates that despite having the same groups being presented at the hydrogen bonding face subtle changes of structure can have significant

effects that alter the analogue's ability to act as a substrate. These effects are not yet predictive. Both the pyrrole mono and dicarboxamide analogues are also degenerate in that they will substitute for all the natural bases with varying degrees of efficiency e.g. the pyrrole-3,4-dicarboxamide will replace A and C and extension is then possible from there but it will also replace T and G and act as a terminator.

When a linker group and reporter have been introduced into the 4 position of the base analogue pyrrole-3-carboxamide a significant reduction in its ability to act as a substrate was observed. Similar results have been observed in comparable systems upon modification of the pyrrole-3,4-dicarboxamide.

The nucleotide analogues of this invention have several advantages over those described above for enzymatic incorporation. The analogues act in a non-degenerate manner and when X = CH are an excellent specific T replacement. Further when a linker arm and reporter group are present they are still good substrates for enzymatic incorporation, acting in a specific manner, see example 2.

Direct enzymatic incorporation is but one aspect of enzymatic recognition and tolerance that has to be considered. The attachment of a linker arm or the analogue itself can effect the ability of an enzyme to either extend from the analogue or read through the analogue and these properties to date are not predictive. The base analogue difluorotoluene causes a pause on read through by an enzyme placing a base opposite in the growing complimentary strand (Proc. Natl. Acad. Sci. USA (1997), 94, 10506-11). The universal base 5-nitroindole (WO 97/28176) when included in an oligo is readily read through by an enzyme and a base replaced opposite. As the 5-nitroindole is a universal base there is likely to be uncertainty as to the base placed opposite during the formation of the complimentary strand to the template.

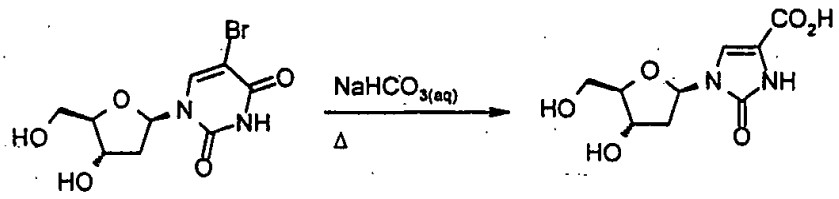
The nucleotide analogues of this invention have advantages in that as phosphoramidites they can be selectively placed in a position

within a DNA oligo via chemical synthesis. Once in that position the presence of a linker and reporter group has been demonstrated to permit an enzyme to read through it and place a base opposite the analogue, see examples 4A-4G. In a comparable experiment with a universal base such as 5-nitroindole the introduction of a linker and reporter was found to have detrimental effects on read through ability. In addition further experiments have shown that the enzyme still treats the invention analogue as a specific base replacement for T by placing an A base opposite the analogue in the growing complimentary strand, see examples 5A-5C. The combined properties permit selective introduction of a reporter group at defined positions that allow the generation of a complimentary strand with unambiguous sequence.

## DETAILED DESCRIPTION OF THE INVENTION

### Example 1

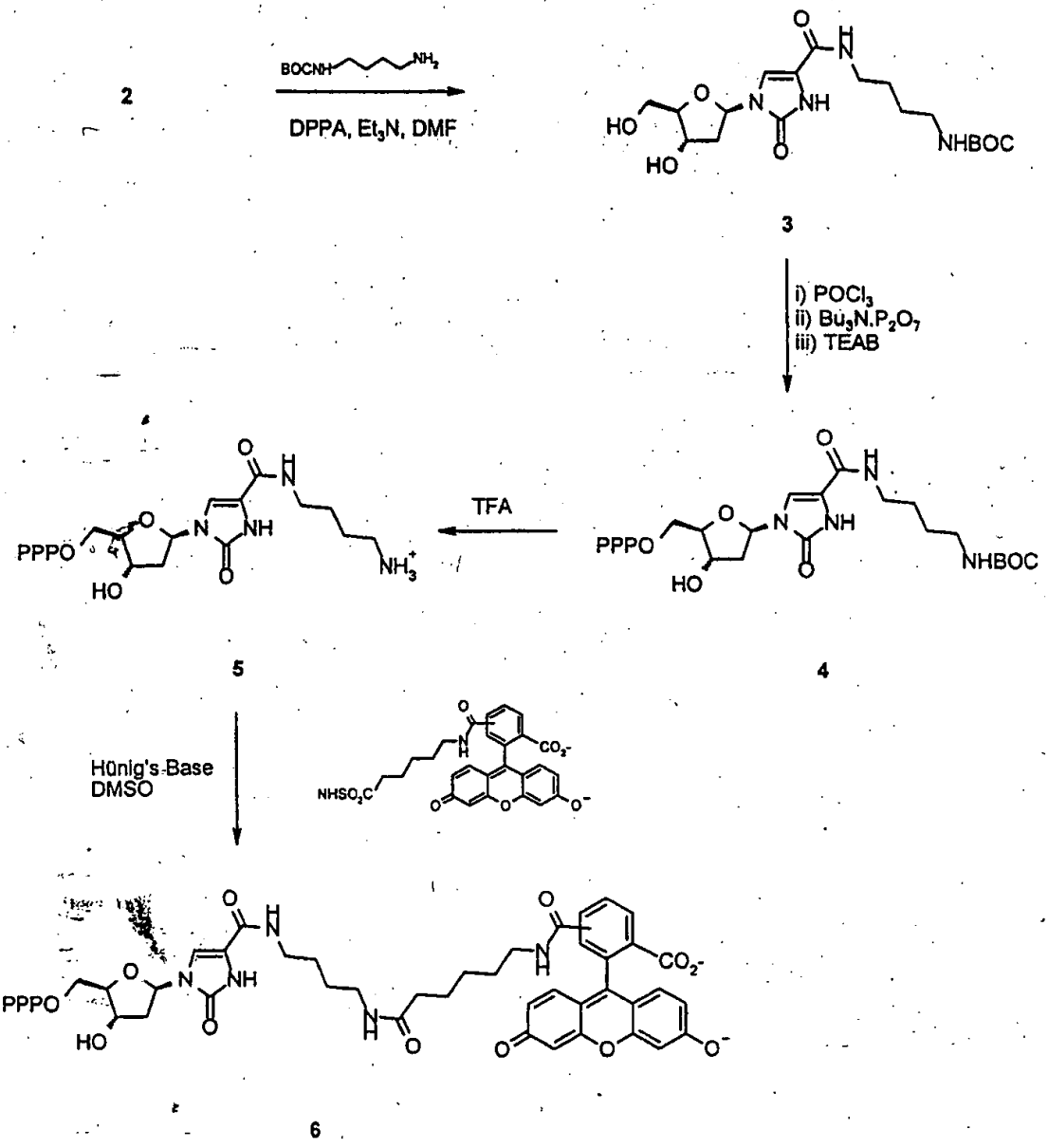
### Preparation of 1-(2'-deoxyribos-1'-yl)imidazolidin-2-one-4-carboxylic acid (2)




1 2

This was carried out according to the method of Otter *et al.* (J Org Chem, 1969, **34**, 1390). The acid was purified by reversed phase HPLC.

$^1$H nmr ($D_2O$) 2.20 (1H, m, 2'-$CH_aH_b$), 2.34 (1H, m, 2'-$CH_aH_b$), 3.50 (2H, m, 5'-$CH_2OH$), 3.86 (1H, m, 4'-CH), 4.33 (1H, m, 3'-CH), 5.87 (1H, t, J = 7 Hz, 1'-CH), 6.97 (1H, s, Ar-H).


2
BOCNH
NH2
DPPA, Et3N, DMF
HO
HO
NHBOC
3
i) POCl3
ii) Bu3N.P2O7
iii) TEAB
PPPO
HO
TFA
NH3+
5
4
Hünig's Base
DMSO
NHSO2C
CO2-
6

**Preparation of 4''-(N'-*tert*-but xycarbonylamino)butyl 1-(2'-deoxyribos-1'-yl)imidazolidin-2-one-4-carboxamide (3)**

The carboxylic acid (30 mg, 0.12 mmol) and 4-(N-*tert*-butoxycarbonylamino)-1-aminobutane (25 mg, 0.14 mmol) in dry DMF (1.5 ml) under a nitrogen atmosphere were treated with a solution of diphenylphosphoryl azide (39 mg, 0.14 mmol) in dry DMF (0.5 ml) and then dry triethylamine (0.04 ml). The mixture was allowed to stir at room temperature for 60 hours. The solvent was removed *in vacuo* to give a pale yellow solid that was purified by preparative tlc (RP18, 1:1 ethanol:water) then reversed phase HPLC to give 18.6 mg of the desired amide as a colourless oil.

$^1$H nmr (CD3OD) 1.42 (9H, s, C(C$\underline{H}_3$)), 1.55 (4H, m, BOCNHCH$_2$C$\underline{H}_2$C$\underline{H}_2$), 2.23 (1H, m, 2'-CH$_a\underline{H}_b$), 3.05 (2H, t, J = 6.6 Hz, BOCNHC$\underline{H}_2$), 3.30 (2H, obscured t, C$\underline{H}_2$NHC(=O)), 3.69 (2H, m, 5'-C$\underline{H}_2$OH), 3.88 (1H, m, 4'-C$\underline{H}$), 4.38 (1H, m, 3'-C$\underline{H}$), 5.99 (1H, t, J = 6.4 Hz, 1'-C$\underline{H}$), and 7.32 (1H, s, Ar-$\underline{H}$).

**Preparation of 4''-(N'-*tert*-butoxycarbonylamino)butyl 1-(2'-deoxyribos-1'yl)imidazolidin-2-one-4-carboxamide-5'-triphosphate (4)**

The nucleoside (3) (18.4 mg, 0.04 mmol) was dissolved in a 1:1 mixture of trimethylphosphate and triethylphosphate (2 ml) under an atmosphere of argon. The mixture was cooled to 0°C with an ice bath and phosphoroyl chloride (17 µl) was added dropwise and the mixture was stirred at 0°C for 2 hours. Tributylammonium pyrophosphate (0.44 ml of a 0.5M solution in dry DMF) was added, followed immediately by addition of tributylamine (50 µl). The mixture was stirred at room temperature for 15 minutes and the reaction was quenched by addition of 1M triethylammonium bicarbonate (5 ml). The mixture was stirred for 1 hour and then the solvents were removed *in vacuo*. The mixture was purified by ion exchange chromatography and then reversed phase chromatography to give a colourless solid. $\lambda_{max}$ ($H_2O$) 264 nm, $^1$Hnmr and $^{31}$Pnmr ($D_2O$) were

consistent with the desired material, but showed that the compound was contaminated with triethylammonium pyrophosphate.

**Preparation of 4''-aminobutyl 1-(2'-deoxyribos-1'-yl)imidazolin-2-one-4-carboxamide-5'-triphosphate trifluoroacetate salt (5)**

The *tert*-butoxycarbonyl protected amine (6.6 μmol) was treated with trifluoroacetic acid (1 ml) at room temperature for 1.5 hours. The solvent was removed *in vacuo* to give the ammonium salt as a colourless solid. $^1$H nmr showed the absence of the 9 proton singlet for the *tert*-butoxycarbonyl group and was otherwise consistent with the desired structure.

**Preparation of 4''-(6'''-(fluorescein-5''''-(and 6'''')-carboxamidohexanamido)butyl 1-deoxyribos-1'-yl)imidazolin-2-one-4-carboxamide-5'-triphosphate (6)**

The amine salt (**5**) was dissolved in anhydrous DMSO and treated with N,N-diisopropyethylamine (5 μl) and 6-(fluorescein-5-(and 6-) carboxamidohexanoic acid NHS ester (3.6 mg). The mixture was allowed to stir for 20 hours and the mixture was purified by ion-exchange chromatography. $\lambda_{max}$ 486 nm, $^1$H nmr was consistent with expected structure.

**Example 2**

A primer extension assay was used to evaluate compounds **(4, 5 and 6)** as a substrate for exonuclease free Klenow fragment DNA polymerase I (EFK). The assay used a $^{33}$P 5' end labelled 15mer primer hybridised to a 24mer template. The sequences of the primer and template are:

| | |
|---|---|
| Primer | 5' TGCATGTGCTGGAGA 3' |
| Template 1 | 3' ACGTACACGACCTCTGAACTAGTC 5' |
| Template 2 | 3' ACGTACACGACCTCTTGGCTAGTC 5' |

One picomole $^{33}P$ labelled primer was hybridised to 2 picomoles of template in x2 Klenow buffer. To this was added either 4 μM dNTPαS or 40μM **(4 or 5)**, 20μM **(6)** or 160μM **(4 or 5)** or a mixture of 4 μM dNTPαS and 40μM **(4 or 5)** or 160μM **(4 or 5)**. One unit EFK and 2 mU **(4 or 5)** or 20 mU **(6)** inorganic pyrophosphatase were used per reaction. Primer alone, primer plus template plus enzyme, , primer plus template plus enzyme plus 4 μM dNTPαS controls were also carried out. The reactions were incubated at 37°C for 3 minutes **(4 and 5)** or 10 minutes **(6)**. Reactions were then stopped by the addition of formamide / EDTA stop solution. Reaction products were separated on a 19% polyacrylamide 7M urea gel. After exposure to Kodak Biomax autoradiography film the incorporation of the analogue was studied by comparison to the control reactions using either primer alone or primer plus template plus enzyme and 4 μM dNTPαS.

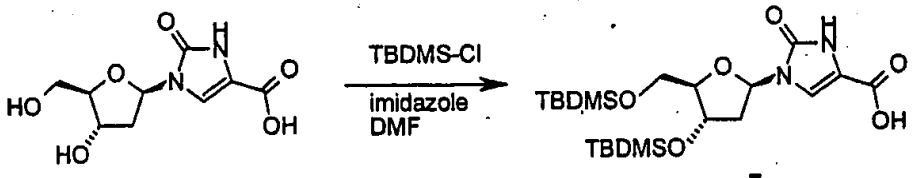
This showed that compounds **(4, 5 and 6)** were good substrates for EFK and that each was incorporated in place of dTTP against Template 1 above. No incorporation in place of dCTP was seen on either template.

## EXAMPLE 3

### Synthesis of phosphoramidites of imidazolidin-2(3*H*)-one-4-carboxamides.

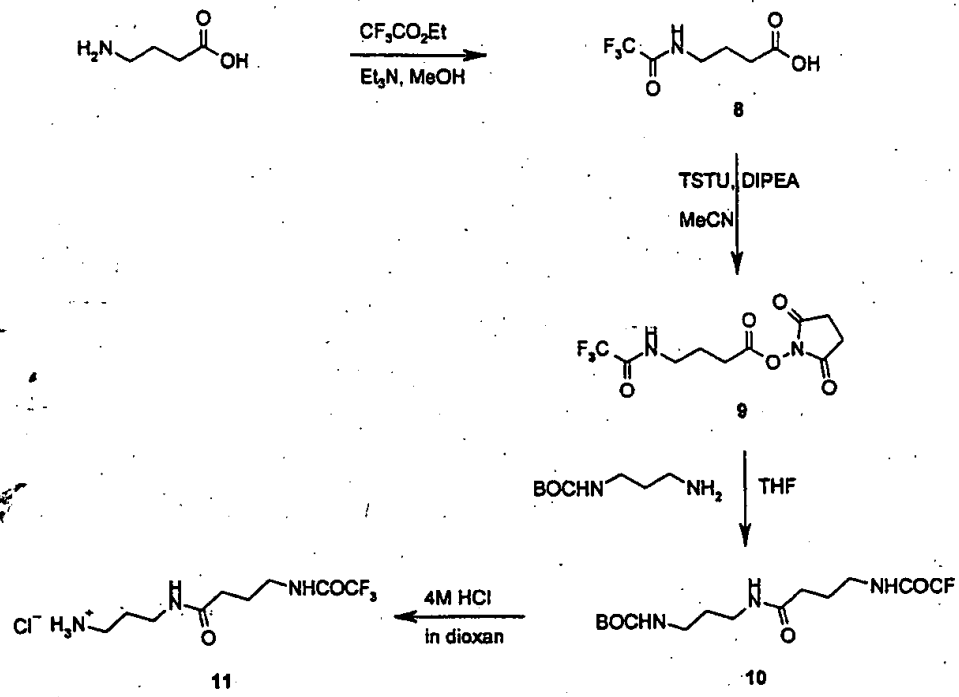
Synthetic Schemes:

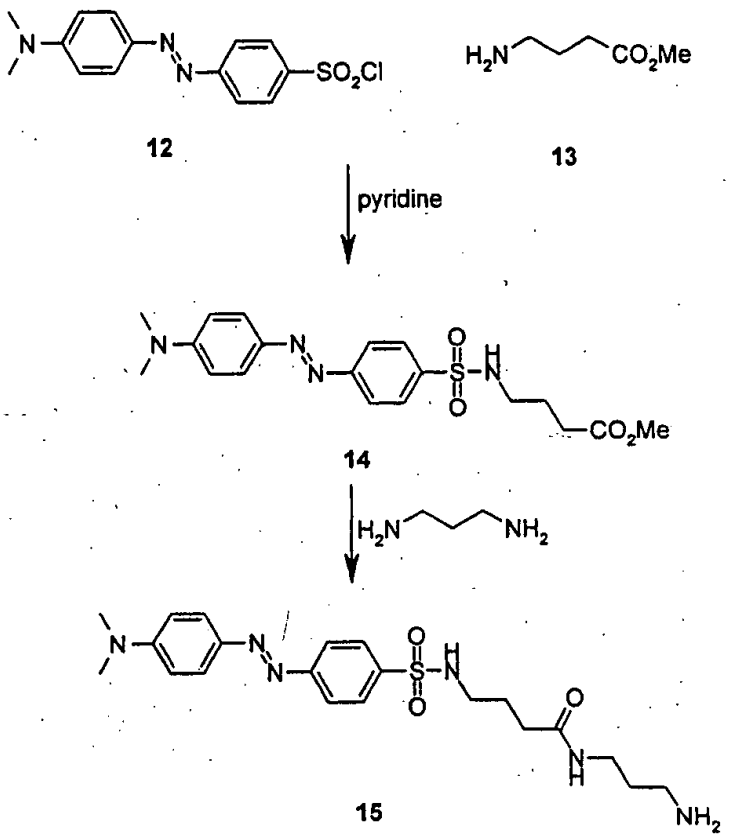
**Preparation of imidazolidin-2(3*H*)-one-4-carboxylic acid nucleoside**

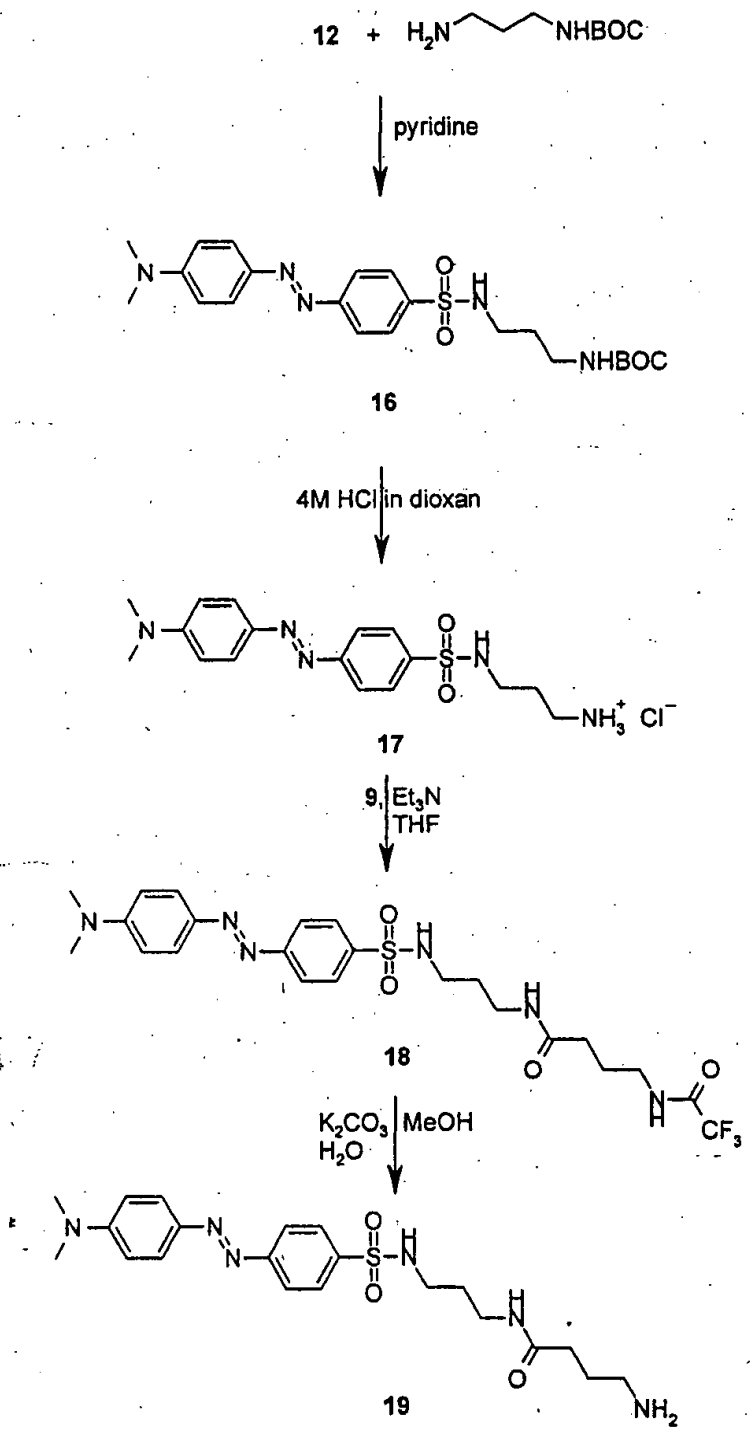


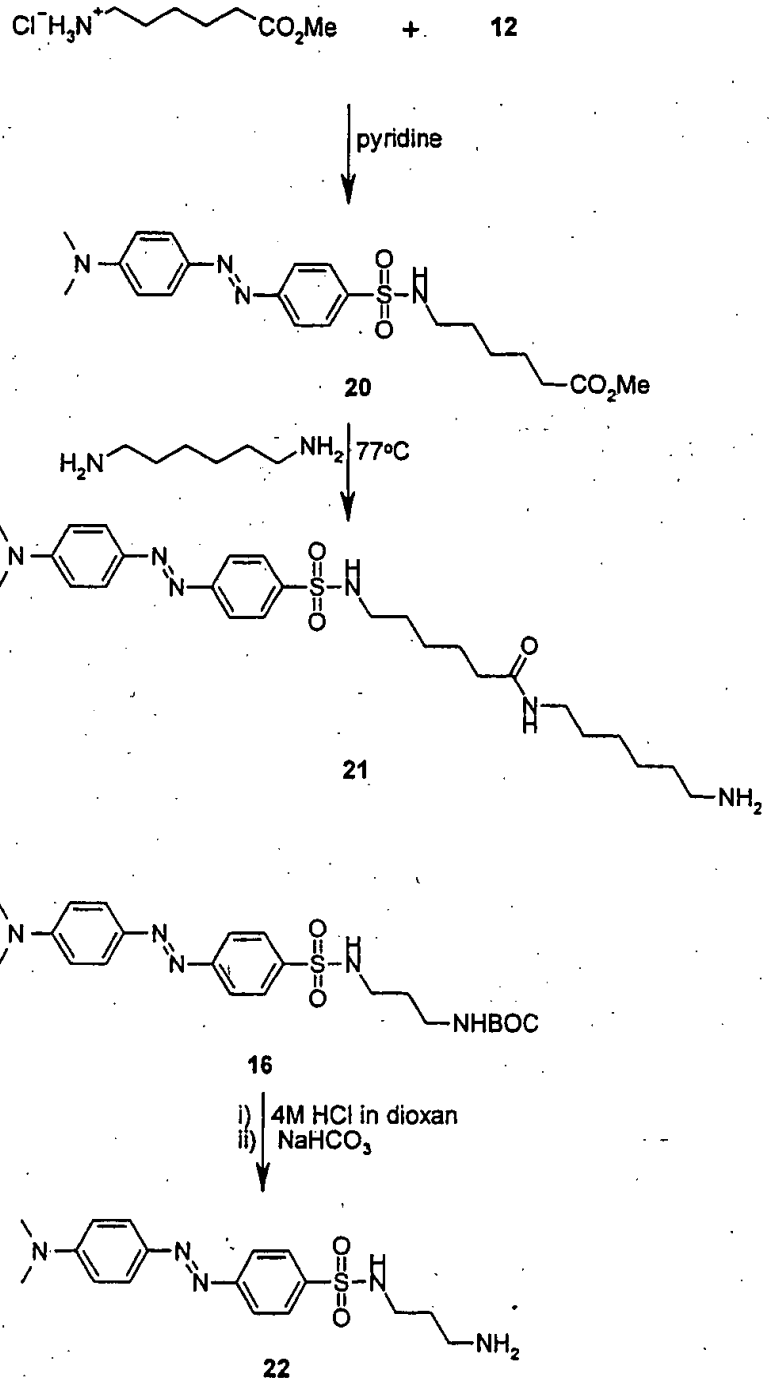

**2** **7**

## Preparation of amines for coupling with imidazolidin-2(3*H*)-one-4-carboxylic acid nucleoside

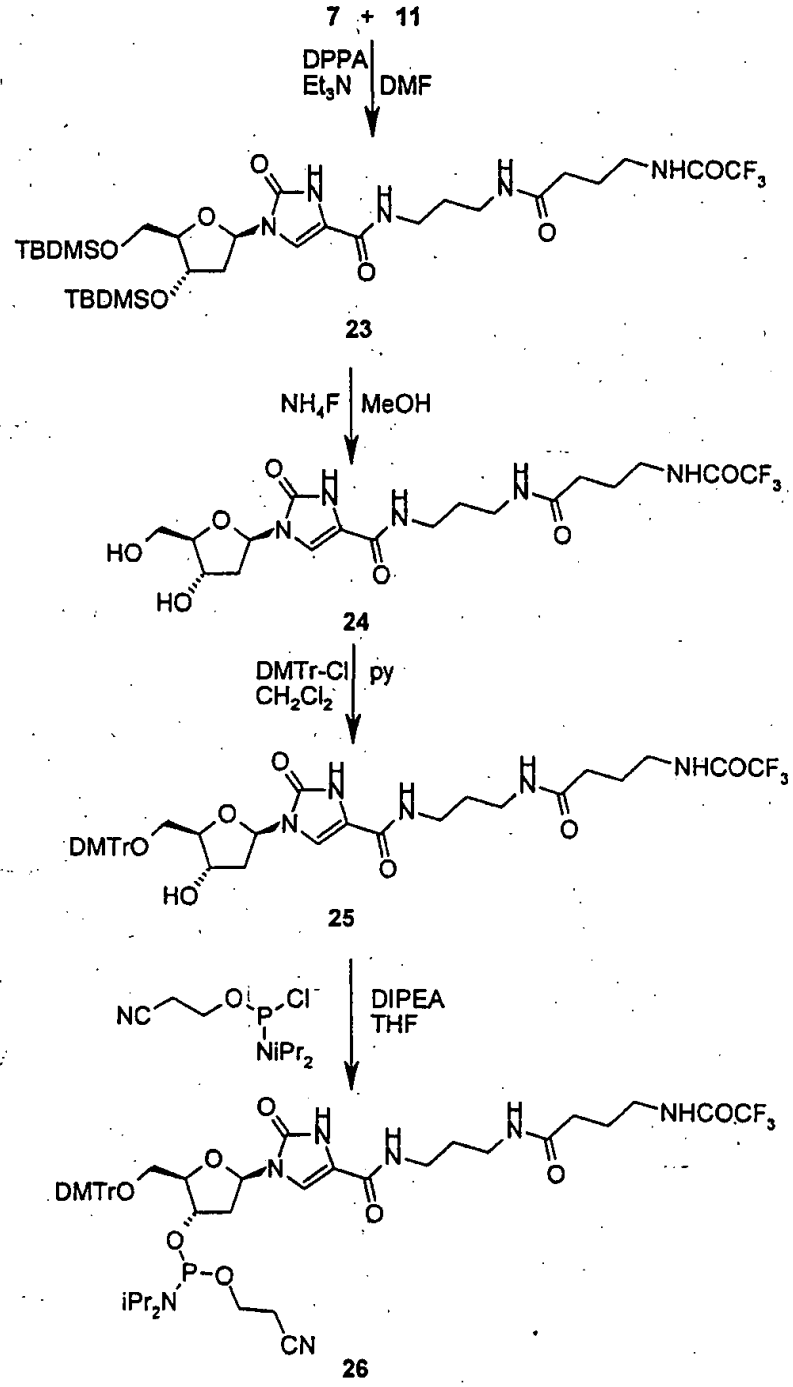



SO2Cl
H2N
CO2Me
12
13
pyridine
CO2Me
14
H2N
NH2
15
NH2

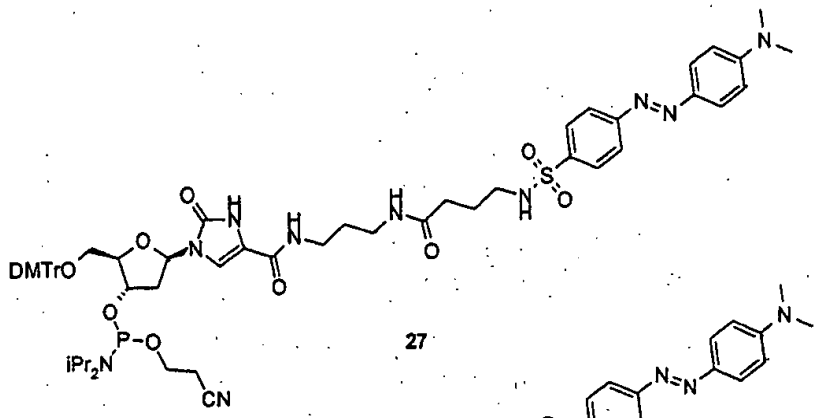

12 + H2N NHBOC
pyridine
NHBOC
16
4M HCl in dioxan
NH3+ Cl−
17
9, Et3N
THF
18
CF3
K2CO3 MeOH
H2O
19
NH2



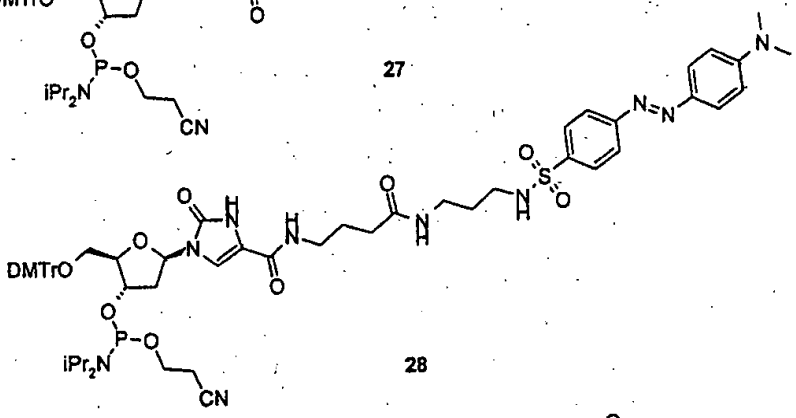

**Example of synthetic steps required to transform amines and acid (2) to phosphoramidites for olig nucleotide synthesis**



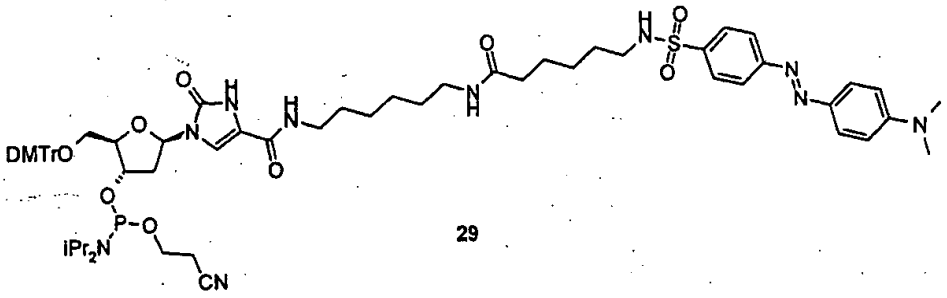

**Structures of other Phosphoramidites Prepared**



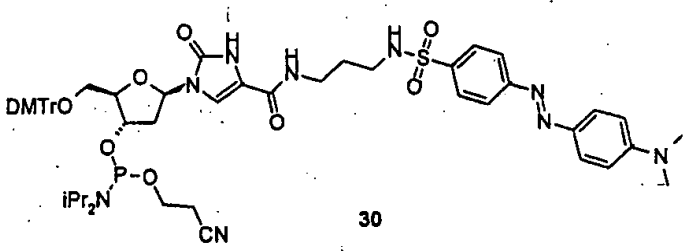

27




28




29




30

5

**Preparation of 4-carboxy-1-(2'-deoxy-3', 5'-di(*tert*-butyldimethylsilyloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one (7)**

The crude reaction mixture from the preparation of the acid (**2**), carried out in a 33 mmol scale, was suspended in dry DMF (50 ml) and treated with imidazole (10.2 g, 150 mmol) and *tert*-butyldimethylsilyl chloride (11.3 g, 75 mmol) and the mixture was stirred at room temperature for an hour. TLC (1:1 methanol:water, on RP18 tlc plates) showed some starting material remained. Further aliquots of *tert*-butyldimethylsilyl chloride were added until the starting material was consumed. The mixture was then heated at 60°C for 16 hours. The mixture was poured into water (200 ml) and then extracted with ethyl acetate (3 x 200 ml), the combined extracts were washed with 2M sodium hydroxide solution and then dried ($MgSO_4$), filtered, evaporated and chromatographed, the desired material was eluted with 10% methanol in dichloromethane to give the silyl ether (3.7 g, 25% in two steps from 5-bromouridine) as a yellow foam. $\delta_H$ ($D_6$-DMSO) 0.07 (6H, s, $Si(C\underline{H}_3)_2$), 0.09 (6H, s, $Si(C\underline{H}_3)_2$), 0.88 (18H, s, 2 x $C(C\underline{H}_3)_3$), 1.98 (1H, m, 2'-$CH_a\underline{H}_b$), 2.34 (1H, m, 2'-$C\underline{H}_aH_b$), 3.62 (2H, m, 5'-$C\underline{H}_2$), 3.71 (1H, br m, 4'-C<u>H</u>), 4.38 (1H, br s, 3'-C<u>H</u>), 5.83 (1H, br t, J = 7.2 Hz, 1'-C<u>H</u>), and 7.07 (1H, s, 5-C<u>H</u>); m/z 471.32 (M-1)$^-$.

**Preparation of 4-trifluoroacetamidobutanoic acid (8)**

4-Aminobutanoic acid (5.16 g, 50 mmol) was suspended in methanol (50 ml) and then treated with ethyl trifluoroacetate (7.81 g, 55 mmol) and triethylamine (5.57 g, 55 mmol). The solid gradually went into solution over 1.5 hours and the mixture was stirred for a further 0.5 hours. The solvent was removed *in vacuo* and the residue was taken into ethyl acetate and washed with 2M hydrochloric acid and brine. The organics were dried ($MgSO_4$), filtered and evaporated to give the desired amide as a colourless solid (8.15 g, 82%). $\nu_{max}$ (film) 3302, 2921, 2853, and 1703; $\delta_H$ (

$CD_3OD$) 1.82 (2H, app quintet, J = 7Hz, $CH_2C\underline{H}_2CH_2$), 2.31 (2H, t, J = 7 Hz, $C\underline{H}_2CO$), 3.32 (2H, m, $C\underline{H}_2NH$), and 9.20 (1H, br s, N<u>H</u>); $\delta_C$ ($CD_3OD$) 25.11, 31.91, 40.11, 117.52, 159.10, and 176.63; m/z 198.13 $(M-1)^-$.

**Preparation of O(N-Succinimidyl) 4-trifluoroacetamidobutanoate (9)**

4-Trifluoroacetamidobutanoic acid (2.1 g, 10.5 mmol) and O-(N-succinimidyl)-N,N,N'N'-tetramethyluronium tetrafluoroborate (3.2 g, 11 mmol) in anhydrous acetonitrile (25 ml) under nitrogen were treated with N,N-diisopropylethylamine (1.42 g, 11 mmol). The mixture was stirred at room temperature for 16 hours. The solvent was removed *in vacuo* and the residue was chromatographed in ether:ethyl acetate to give the desired ester as a colourless solid (2.66 g, 90%). $\nu_{max}$ (nujol) 3344, 1811, 1776, and 1721 $cm^{-1}$; $\delta_H$ ($CDCl_3$) 2.06 (2H, quintet, J = 6.9 Hz, $CH_2C\underline{H}_2CH_2$), 2.70 (2H, t, J = 6.9 Hz, $C\underline{H}_2CONHS$), 2.86 (4H, s, $COC\underline{H}_2C\underline{H}_2CO$), 3.47 (2H, t, J = 6.9 Hz, $C\underline{H}_2N$), and 7.16 (1H, br s, N<u>H</u>); $\delta_C$ ($CDCl_3$) 23.70, 25.60, 28.30, 38.72, 115.70, 157.70, 168.16, and 169.33; m/z 295.28 $(M-1)^-$.

**Preparation of N-3-(*tert*-butoxycarbonylamino)propyl 4-trifluoroacetamidobutanamide (10)**

O-(N-Succinimidyl) 4-trifluoroacetamidobutanoate (834 mg, 2.8 mmol) in anhydrous tetrahydrofuran (5 ml) under an atmosphere of argon was treated with 3-*tert*-butoxycarbonylamino-1-aminopropane (500 mg, 2.9 mmol). The mixture was stirred at room temperature for 16 hours. The solvent was removed *in vacuo* and the residue was partitioned between ether and water. The organics were washed with 2M hydrochloric acid, and brine, then dried ($MgSO_4$), filtered and evaporated to give the desired amide as a colourless oil that solidified on standing (570 mg, 57%). $\delta_H$ ($CDCl_3$) 1.44 (9H, s, $C(C\underline{H}_3)_3$), 1.62 (2H, br quintet, J = 6.6 Hz, $NCH_2C\underline{H}_2CH_2N$), 1.93 (2H, br quintet, J = 6.0 Hz, $CH_2C\underline{H}_2CH_2CO$), 2.36

(2H, t, J = 6.0 Hz, $C\underline{H}_2CO$), 3.17 (2H, q, J = 6.3 Hz, $CF_3CONHC\underline{H}_2$), 3.29 (2H, q, J = 6.3 Hz, $C\underline{H}_2NHCO_2{}^tBu$), 3.40 (2H, q, J = 5.7 Hz, $C\underline{H}_2NHCOCH_2$), 4.96 (1H, br s, $CH_2N\underline{H}COCH_2$), 7.28 (1H, br s, $CH_2N\underline{H}CO_2{}^tBu$), and 8.49 (1H, br s, $CF_3CON\underline{H}CH_2$).

**Preparation of N-3-aminopropyl 4-trifluoroacetamidobutanamide hydrochloride (11)**

N-(3-*tert*-butoxycarbonylamino)propyl 4-trifluoroacetamidobutanamide (570 mg, 1.6 mmol) was treated with 4M hydrogen chloride in dioxan (5 ml) at room temperature for 1.5 hours. The solvent was removed *in vacuo* and the resulting oil dried *in vacuo* to give the desired amine salt as a thick gum that was used without further purification. $\delta_H$ ($CD_3OD$) 1.79 – 1.90 (4H, m, $NCH_2C\underline{H}_2CH_2N$ and $CH_2C\underline{H}_2CH_2CO$), 2.61 (2H, t, J = 7 Hz, $C\underline{H}_2CO$), 2.95 (2H, br t, J = 7.2 Hz, $CF_3CONHC\underline{H}_2$), and 3.25 – 3.33 (4H, m, $C\underline{H}_2NHCOCH_2$, $C\underline{H}_2N^+H_3$).

**Preparation of methyl 4-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)butanoate (14)**

Anhydrous pyridine (5 ml) was added to a mixture of methyl 4-aminobutanoate (0.43 g, 3.1 mmol) and 4-(4-N,N-dimethylaminophenyl)azobenzenesulfonyl chloride (1 g, 3.1 mmol) under an atmosphere of argon. The mixture was stirred at room temperature for 1 hour. The solvent was removed *in vacuo*, and the solid was partitioned between ethyl acetate and pH 6 citrate buffer. The organics were separated, and the aqueous layer was extracted with ethyl acetate (2 x 100 ml). The combined extracts were dried ($MgSO_4$), filtered and evaporated to give the desired sulfonamide as an orange solid (1.06 g, 80%). $\lambda_{max}$ (EtOH) 434 nm; $\delta_H$ ($CDCl_3$) 1.82 (2H, tt, J = 7.0 and 6.6 Hz, $CH_2C\underline{H}_2CH_2$), 2.37 (2H, t, J = 7.0 Hz, $CH_2C\underline{H}_2CO$), 3.05 (2H, app q, J = 6.6 Hz), 3.13 (6H, s,

$(C\underline{H}_3)_2N$), 3.66 (3H, s, $CO_2C\underline{H}_3$), 4.73 (1H, t, J = 6.2 Hz, N<u>H</u>), 6.76 (2H, d, J = 9.1 Hz, 2 x Ar<u>H</u>), 7.91 (2H, d, = 9.1 Hz, 2 x Ar<u>H</u>), 7.92 (2H, d, J = 9.1 Hz, 2 x Ar<u>H</u>), and 7.95 (2H, d, J = 9.1 Hz, 2 x Ar<u>H</u>); $\delta_C$ ($CDCl_3$) 24.67, 30.96, 40.30, 42.64, 51.81, 111.48, 122.64, 125.75, 128.04, 139.30, 143.60, 153.14, 155.67, and 173.59; m/z 405.36 $(M + 1)^+$.

**Preparation of N-3-(N'-*tert*-butoxycarbonyl)aminopropyl 4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamide (16)**

This sulfonamide was prepared in an analogous fashion to 14, using 4-(4-N,N-dimethylaminophenyl)azobenzenesulfonyl chloride (1 g, 3.1 mmol) and mono(*tert*-butoxycarbonyl)-1,3-propanediamine (0.49 g, 2.8 mmol. The sulfonamide (0.82 g, 63%) was isolated as an orange solid. $\delta_H$ ($CDCl_3$) 1.39 (9H, s, $C(C\underline{H}_3)_3$), 1.61 (2H, quintet, J = 6 Hz, $CH_2C\underline{H}_2CH_2$), 3.01 (2H, q, J = 6 Hz, $C\underline{H}_2NHSO_2$), 3.12 (6H, s, $(C\underline{H}_3)_2N$), 3.19 (2H, q, J = 6 Hz, $C\underline{H}_2NHBOC$), 4.63 (1H, br t, $N\underline{H}SO_2$), 5.70 (1H, br t, N<u>H</u>BOC), 6.77 (2H, d, J = 9 Hz, 2 x Ar<u>H</u>), and 7.89 – 8.01 (6H, m, 6 x Ar<u>H</u>); $\delta_C$ ($CDCl_3$) 28.33, 30.53, 36.77, 39.81, 40.31, 79.81, 111.50, 122.58, 125.72, 127.96, 139.92, 143.61, 153.10, and 155.47; m/z 460.43 $(M-1)^-$.

**Preparation of Methyl 6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanoate (20)**

This sulfonamide was prepared in an analogous fashion to **14**, using 4-(4-N,N-dimethylaminophenyl)azobenzenesulfonyl chloride (1.03 g, 3.2 mmol) and methyl 6-aminohexanoate hydrochloride salt (0.58 g, 3.2 mmol) giving the sulfonamide (810 mg, 59%) as an orange solid. $\lambda_{max.}$ (MeOH) 536 nm; $\delta_H$ ($D_6$-DMSO) 1.22 (2H, m, $C\underline{H}_2$), 1.31 – 1.48 (4H, m, 2 x $C\underline{H}_2$), 2.22 (2H, t, J = 7.2 Hz, $C\underline{H}_2CO$), 2.74 (2H, t, J = 7.2 Hz, $C\underline{H}_2SO_2$), 3.08 (6H, s, $(C\underline{H}_3)_2N$), 3.54 (3H, s, $CO_2C\underline{H}_3$), 6.85 (2H, d, J = 9.6 Hz, 2 x

ArH), 7.82 (2H, d, J = 9.6 Hz, 2 x ArH), and 7.89 (4H, s, $N_2C_6H_4SO_2$); $\delta_C$ ($D_6$-DMSO) 23.89, 25.46, 28.57, 33.08, 42.24, 51.13, 111.59, 122.18, 125.36, 127.36, 142.59, 153.10, 154.44, and 173.20; m/z 431 $(M-1)^-$.

**Preparation of N-3-aminopropyl 4-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)butanamide (15)**

Methyl 4-(4-(4-dimethylaminophenyl)azobenzenesulfonamido)butanoate (1 g, 2.47 mmol) was dissolved in 1,3-diaminopropane (10ml) and the mixture was heated to 100°C under nitrogen for 3 hours. The solvent was removed *in vacuo* and the solid was partitioned between pH 6 citrate buffer and chloroform. The organics were dried ($MgSO_4$), filtered and evaporated to give an orange solid (0.95 g, 86%); $\lambda_{max}$ (MeOH) 438 nm; $\delta_H$ ($D_6$-DMSO) 1.53 (4H, m, $NHCH_2CH_2CH_2N$, $NHCH_2CH_2CH_2CO$), 2.16 (2H, t, J = 7.3 Hz, $CH_2CO$), 2.75 (2H, t, J = 7 Hz, $CH_2N$), 2.81 (2H, t, J = 7 Hz, $CH_2N$), 3.13 (6H, s, $N(CH_3)_2$), 3.78 (2H, t, J = 6.6 Hz, $CH_2NHCO$), 6.81 (2H, d, J = 9.6 Hz, 2 x ArH), 7.84 (2H, d, J = 9.6 Hz, 2 x ArH), 7.90 (4H, s, 4 x ArH), and 8.12 (1H, s, NH); m/z 447.29 $(M+1)^+$.

**Preparation of N-6-aminohexyl 6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanamide (21)**

Methyl 6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanoate (0.72 g, 1.7 mmol) was added to molten, stirred 1,6-diaminohexane (10.7 g, 92 mmol) at 50°C. The mixture was heated at 77°C for 6 hours and then the mixture was allowed to cool to room temperature. The mixture was then melted and poured into pH 6 citrate buffer and the solid extracted with chloroform, the extracts were washed with water (3 x 150 ml), then pH 6 citrate buffer, dried (MgSO4), filtered and evaporated. The resultant solid was suspended

in chloroform/pH 6 buffer, and filtered off. The solid was washed with water and air dried to give the amine (580 mg, 66 %) as an orange solid. $\delta_H$ ($D_6$-DMSO) 1.20 – 1.50 (14H, m, 7 x $C\underline{H}_2$), 2.00 (2H, t, J = 7.2 Hz, $C\underline{H}_2CONH$), 2.52 (2H, br m, $C\underline{H}_2N$), 2.76 (2H, t, J = 7.2 Hz, $C\underline{H}_2N$), 3.00 (2H, br q, $C\underline{H}_2N$), 3.11 (6H, s, $(C\underline{H}_3)_2N$), 6.88 (2H, d, J = 9 Hz, 2 x Ar$\underline{H}$), 7.75 (1H, br s, N$\underline{H}$), 7.85 (2H, d, J = 9 Hz, 2 x Ar$\underline{H}$), and 7.92 (4H, s, $N_2C_6\underline{H}_4SO_2$); $\delta_C$ ($D_6$-DMSO) 24.84, 25.72, 25.85, 26.12, 28.77, 29.05, 35.24, 38.25, 42.42, 45.55, 122.17, 125.36, 127.75, 140.35, 142.60, 153.10, 154.34, and 171.70; m/z 517 $(M+1)^+$.

**Preparation of N-3-aminopropyl 4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamide (22)**

N-(3-N'-(*tert*-butoxycarbonyl)aminopropyl) 4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamide (1.1 g, 2.4 mmol) in dichloromethane (10 ml) was treated with 4M hydrogen chloride in 1,4-dioxan (4 ml) at room temperature. The mixture was stirred for 16 hour and the solvent removed *in vacuo*. The residue was treated with saturated sodium bicarbonate and the resulting solid dissolved in ethanol. The ethanolic solution was evaporated and dried *in vacuo* to give the amine (0.86 g, quantitative) as an orange solid. $\delta_H$ ($D_6$-DMSO) 1.34 (2H, m, $CH_2C\underline{H}_2CH_2NH_2$), 2.75 (2H, br t, $C\underline{H}_2NHSO_2$), 2.88 (2H, m, $C\underline{H}_2NH_2$), 3.08 (6H, s, $(C\underline{H}_3)_2N$), 6.85 (2H, d, J = 9.3 Hz, 2 x Ar$\underline{H}$), 7.79 (2H, d, J = 9.3 Hz, 2 x Ar$\underline{H}$), 8.84 (2H, d, J = 6.3 Hz, 2 x Ar$\underline{H}$), and 7.89 (2H, d, J = 6.3 Hz, 2 x Ar$\underline{H}$); m/z 363.51 $(M+1)^+$

**Preparation of N-3-aminopropyl 4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamide hydrochloride (17)**

N-(3-N'-(*tert*-butoxycarbonyl)aminopropyl) 4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamide (0.82 g, 1.78 mmol) was

suspended in 1,4-dioxan (3 ml) and treated with 4M hydrogen chloride in 1,4-dioxan (2 ml). The mixture was allowed to stir at room temperature for 16 hours. The solvent was removed *in vacuo* and the resulting gum was co-evaporated with ethanol and dried *in vacuo*. The amine hydrochloride was used without further purification. $\delta_H$ ($CD_3OD$) 1.85 (2H, quintet, $CH_2C\underline{H}_2CH_2$), 3.00 (4H, q, 2 x $C\underline{H}_2N$), 3.35 (6H, s, $(C\underline{H}_3)_2N$), 7.16 (2H, d, J = 9 Hz, 2 x Ar$\underline{H}$), and 7.90 – 7.99 (6H, m, 6 x Ar$\underline{H}$); $\delta_C$ ($CD_3OD$) 29.30, 37.91, 38.35, 41.01, 42.17, 116.57, 121.40, 129.51, 130.48, 140.59, and 157.59; m/z 362 ($M^+$).

**Preparation of N-3-(N-4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propyl 4-trifluoroacetamidobutanamide (18)**

Unpurified N-(3-aminopropyl) 4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamide hydrochloride (1.78 mmol) and O-(N-succinimidyl) 4-trifluoroacetamidobutanoate (0.63 g, 2.1 mmol) were suspended in anhydrous tetrahydrofuran (20 ml) under an atmosphere of nitrogen at room temperature. The mixture was treated with triethylamine (0.36 g, 3.6 mmol) and stirred for 4 hours a further aliquot of O-(N-succinimidyl) 4-trifluoroacetamidobutanoate (0.2 g, 0.6 mmol) was added and the mixture was stirred for 1 hour and the solvent was then removed *in vacuo*. The residue was chromatographed, eluting the desired amide in ethyl acetate and then 5 % methanol in dichloromethane accompanied by elution of N-hydroxysuccinimide. The solid was taken into ethyl acetate and washed with pH 6 citrate buffer, dried ($MgSO_4$), filtered and evaporated to give the desired amide (690 mg, 65 %) as an orange powder. $\lambda_{max.}$ (MeOH) 438 nm; $\delta_H$ ($D_6$-DMSO) 1.48 (2H, quintet, J = 7 Hz, $NCH_2C\underline{H}_2CH_2N$), 1.63 (2H, quintet, J = 7.4 Hz, $NCH_2C\underline{H}_2CH_2CO$), 2.01 (2H, t, J = 7.4 Hz, $CH_2CO$), 2.73 (2H, br t, $C\underline{H}_2NSO_2$), 2.97 (2H, br t, $CONHC\underline{H}_2$), 3.06 (6H, s, $(C\underline{H}_3)_2N$), 3.11 (2H, br t, $C\underline{H}_2NHCOCF_3$), 6.82 (2H,

d, J = 9.1 Hz, 2 x ArH), 7.64 (1H, br t, J = 5.9 Hz, $NHSO_2$), 7.80 (2H, d, J = 9.1 Hz, 2 x ArH), 7.87 (4H, s, $N_2C_6H_4SO_2$), and 9.40 (1H, br t, NH); $\delta_C$ ($CD_3OD$) 26.42, 31.19, 34.27, 37.77, 113.45, 117.80 (q, J = 289 Hz, $CF_3$), 124.11, 127.29, 142.00, 142.09, 155.00, 156.37, 158.06 (q, J = 36 Hz, $COCF_3$), and 173.32; m/z 543.50 ($M^+$).

**Preparation of N-3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonyl)aminopropyl 4-aminobutanamide (19)**

N-3-(N-4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propyl 4-trifluoroacetamidobutanamide (0.68 g, 1.25 mmol) in methanol:water (1:1) (40 ml) under nitrogen was treated with potassium carbonate. The mixture was heated at 60°C for 3 hours. The solvent was removed *in vacuo*. The residue was washed with chloroform/pH 6 citrate buffer and then ethanol. The resulting solid was air dried and the combined with the ethanol washings to give an orange solid (0.54 g, 96 %). $\delta_H$ ($D_6$-DMSO)1.51 (2H, quintet, J = 6.9Hz, $NHCH_2CH_2CH_2NH_2$), 1.59 (2H, quintet, J = 7.2 Hz, $CH_2CH_2CH_2CO$), 2.07 (2H, t, J = 7.2 Hz, $CH_2CO$), 2.57 (2H, t, J = 6.9 Hz, $CH_2NHSO_2$), 2.77 (2H, t, J = 6.9 Hz, $CH_2N$), 2.79 (2H, br m, $CH_2N$), 3.01 (6H, s, $(CH_3)_2N$), 6.86 (2H, d, J = 9.9 Hz, 2 x ArH), 7.84 (2H, d, J = 9.9Hz, 2 x ArH), and 7.91 (4H, s, $N_2C_6H_4SO_2$); $\delta_C$ ($D_6$-DMSO) 29.24, 29.33, 32.95, 35.77, 35.89, 40.99, 111.59, 122.20, 125.37, 127.77, 140.16, 142.60, 153.11, 154.47, and 172.20; m/z 447.38 $(M+1)^+$.

**Preparation of N-3-(4-trifluoroacetamidobutanoyl)aminopropyl 1-(3,'5'-di(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (23)**

1-(3,'5'-di(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxylic acid (628 mg, 1.3 mmol) and N-(3-aminopropyl) 4-trifluoroacetamidobutanamide hydrochloride (388 mg, 1.3 mmol) in anhydrous N,N-dimethylformamide (5 ml) under an atmosphere of argon were treated with triethylamine (263 mg, 2.6 mmol) and diphenylphosphoryl azide (385 mg, 1.4 mmol) at room temperature. The mixture was stirred for 16 hours. Diphenylphosphoryl azide (300 mg) was added and the mixture stirred for a further 8 h. The solvent was removed *in vacuo* and the residue taken into ethyl acetate, washed with water, 2M hydrochloric acid and 2M sodium bicarbonate, dried (MgSO4), filtered and evaporated and then chromatographed in 5% methanol in dichloromethane to give the desired material as a pale yellow gum (440 mg, 48%). $\delta_H$ ($CDCl_3$) 0.08 (12H, 2 x s, 2 x $Si(C\underline{H}_3)_2$), 0.88 (18H, 2 x s, 2 x $SiC(C\underline{H}_3)_3$), 1.72 (2H, br m, $NHCH_2C\underline{H}_2CH_2NH$), 1.92 (2H, br m, $CH_2C\underline{H}_2CH_2CO$), 2.20 (2H, br m, 2'-$C\underline{H}_2$), 2.33 (2H, br m, $CH_2CH_2C\underline{H}_2CO$), 3.25 (2H, br m, $CF_3CONHC\underline{H}_2$), 3.37 (4H, br m, 2 x $CONHC\underline{H}_2$), 3.68 (2H, m, 5'-$C\underline{H}_2$), 3.90 (1H, br s, 4'-C<u>H</u>), 4.44 (1H, br s, 3'-C<u>H</u>), 6.03 (1H, t, J = 6.6 Hz, 1'-C<u>H</u>), 7.18 (1H, s, imidazolone 5-<u>H</u>), 7.48 (1H, br s, N<u>H</u>), 8.69 (1H, br s, N<u>H</u>), and 10.54 (1H, br s, N<u>H</u>); $\delta_C$ ($CDCl_3$) –5.51, -5.41, -4.82, -4.74, 17.95, 18.42 95, 24.21, 25.86, 25.94, 29.26, 33.82, 36.64, 39.73, 40.34, 63.43, 72.58, 82.67, 87.55, 113.76, 116.05, 129.85, 152.69, 157.76, 159.48, and 173.92; m/z 708.22 (M-1)$^-$.

**Preparation of N-3-(4-(4-(4-dimethylaminophenyl)azobenzenesulfonamido)butanoyl)aminopropyl 1-(3',5'-bis(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

This amide was prepared according to the same procedure as 23 using 1-(3,'5'-bis(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxylic acid (0.9 g, 1.9 mmol), N-(3-aminopropyl) 4-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)butanamide (0.9 g, 2mmol), triethylamine (0.20 g, 2 mmol) and diphenylphosphoroyl azide (0.61 g, 2.2 mmol) in anhydrous N,N-dimethylformamide (10 ml). The amide (0.82 g, 48 %) was obtained after chromatography, eluting with dichloromethane:methanol (100:5), accompanied by an unidentified impurity as an orange gum. $\delta_H$ ($CDCl_3$) 0.08 (12H, s, 2 x $Si(C\underline{H}_3)_2$), 0.87 (18H, s, 2 x $SiC(C\underline{H}_3)_3$), 1.70 (2H, br m, $NCH_2C\underline{H}_2CH_2N$), 1.83 (2H, br quintet, $CH_2C\underline{H}_2CH_2CO$), 2.20 (2H, m, 2'-$C\underline{H}_2$), 2.29 (2H, t, J = 7.8 Hz, $C\underline{H}_2CO$), 2.98 (2H, m, $C\underline{H}_2SO_2$), 3.10 (6H, s, $N(C\underline{H}_3)_2$), 3.25 (2H, m, $C\underline{H}_2N$), 3.36 (2H, m, $C\underline{H}_2$), 3.70 (2H, m, 5'-$C\underline{H}_2$), 3.90 (1H, br m, 4'-C<u>H</u>), 4.43 (1H, m, 3'-C<u>H</u>), 6.07 (1H, t, J = 7.2 Hz, 1'-C<u>H</u>), 6.46 (0.5H, br t, N<u>H</u>), 6.74 (2H, d, J = 9 Hz, 2 x Ar<u>H</u>), 7.17 (1H, s, imidazole 5-<u>H</u>), 7.88 (2H, d, J = 9 Hz, 2 x Ar<u>H</u>), 7.90 (2H, d, J = 9 Hz, 2 x Ar<u>H</u>), 7.93 (2H, d, J = 9 Hz, 2 x Ar<u>H</u>), and 10.09 (1H, br s, N<u>H</u>); m/z 901.02 ($M^+$).

**Preparation of N-3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propyl 1-(3',5'-bis(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

This amide was prepared according to the same procedure as 23 using 1-(3,'5'-bis(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-

yl)imidazolidin-2(3*H*)-one-4-carboxylic acid (710 mg, 1.5 mmol), N-3-aminopropyl 4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamide (361 mg, 1 mmol), triethylamine (101 mg, 1 mmol) and diphenylphosphoroyl azide (303 mg, 1.1 mmol) in anhydrous N,N-dimethylformamide (10 ml). The amide (280 mg) was obtained by chromatography, eluting with dichloromethane:methanol (100:5) accompanied by an impurity. $\delta_H$ ($CDCl_3$) 0.07 (12H, s, 2 x Si($C\underline{H}_3$)$_2$, 0.88 (9H, s, SiC($C\underline{H}_3$)$_3$), 0.89 (9H, s, SiC($C\underline{H}_3$)$_3$), 1.69 (2H, m, NHCH$_2$C$\underline{H}_2$CH$_2$NH), 2.98 (2H, m, C$\underline{H}_2$NHSO$_2$), 3.11 (6H, s, ($C\underline{H}_3$)$_2$N), 3.33 (2H, m, CONHC$\underline{H}_2$), 3.68 (2H, m, 5'-C$\underline{H}_2$), 3.90 (1H, br s, 4'-C$\underline{H}$), 4.44 (1H, br s, 3'-C$\underline{H}$), 6.02 (1H, t, J = 6.9 Hz, 1'-CH), 6.74 (2H, d, J = 9.3 Hz, 2 x Ar$\underline{H}$), 7.27 (1H, s, imidazolone 5$\underline{H}$), and 7.85 – 7.96 (6H, m, 6 x Ar$\underline{H}$); m/z 816.16 ($M^+$).

**Preparation of N-(4-(3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propylamino)-4-oxobutyl 1-(3',5'-di(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

This amide was prepared according to the procedure used for the preparation of **23** using 1-(3,'5'-bis(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxylic acid (710 mg, 1.5 mmol), N-3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propyl 4-aminobutanamide (446 mg, 1 mmol), triethylamine (101 mg, 1mmol), and diphenylphosphoryl azide (303 mg, 1.1 mmol) in anhydrous N,N-dimethylformamide (10 ml). The product was obtained after chromatography, eluting with 5 % methanol in dichloromethane to give the product as an orange oil. This was taken into dichloromethane (80 ml) and washed with water (2 x 100 ml), dried ($MgSO_4$) and evaporated to give the product as an orange oil (500 mg, 55 %). $\delta_H$ ($CD_3OD$) –0.38 (6H, s, Si($C\underline{H}_3$)$_2$), 0.11 (6H, s, Si($C\underline{H}_3$)$_2$), 0.88 (18H, s, 2 x SiC($C\underline{H}_3$)$_3$), 1.65 (2H, quintet, J = 6.6 Hz, NHCH$_2$C$\underline{H}_2$CH$_2$NH), 1.79 (2H, quintet, J = 6.9 Hz,

$CH_2CH_2CH_2CO$), 2.16 – 2.21 (4H, m, 2'-$CH_2$, $CH_2CO$), 2.89 (2H, t, J = 6.6 Hz, $CH_2NHSO_2$), 3.09 (6H, s, $(CH_3)_2N$), 3.18 – 3.26 (4H, m, 2 x $CH_2NHCO$), 3.69 (2H, m, 5'-$CH_2$), 3.89 (1H, m, 4'-CH), 4.49 (1H, m, 3'-CH), 5.96 (1H, t, J = 6.3 Hz, 1'-CH), 6.68 (2H, d, J = 9.3 Hz, 2 x ArH), 7.18 (1H, s, imidazolone 5H), and 7.84 – 7.94 (6H, m, 6 x ArH); m/z 901.27 ($M^+$).

**Preparation of N-6-(6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanamido)hexyl 1-(2'-deoxy-3',5'-di(*tert*-butyldimethylsilyloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

N-6-aminohexyl 6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanamide (580 mg, 1.1 mmol) and 1-(3,'5'-di(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxylic acid (800 mg, 1.7 mmol) in anhydrous N,N-dimethylformamide (10 ml) were treated with bromotripyrrolidinephosphonium hexafluorophosphate (800 mg, 1.7 mmol) and triethylamine (222 mg, 2.2 mmol) at room temperature under an atmosphere of argon. The mixture was stirred for 64 hours. The solvent was removed *in vacuo* and the reaction mixture chromatographed by presorbing onto silica gel and the desired amide was eluted in 5% methanol in dichloromethane to give the desired material (720 mg) accompanied by 3 molar equivalents of tripyrrollidinephosphoramide. $\delta_H$ ($CDCl_3$) 0.07 (6 H, s, $Si(CH_3)_2$), 0.09 (6 H, s, $Si(CH_3)_2$), 0.87 (9 H, s, $SiC(CH_3)_3$), 0.90 (9 H, s, $SiC(CH_3)_3$), 1.30 (8 H, app t, J = 7.2 Hz, 4 x $CH_2$), 1.40 – 1.54 (6H, m, 3 x $CH_2$), 2.13 (4H, m, 2'-$CH_2$, $CH_2CO$), 2.94 (2H, t, J = 6.3 Hz, $CH_2NHSO_2$), 3.16 (2H, obscured m, $CH_2N$), 3.22 (2H, q, J = 6.3 Hz, $CH_2NHCO$), 3.67 (1H, m, 5'-$CH_aH_b$), 3.80 (1H, m, 5'-$CH_aH_b$), 3.90 (1H, br m, 4'-CH), 4.42, (1H, br m, 3'-CH), 6.10 (1H, t, J = 6.2 Hz, 1'-CH), 6.24 (1H, t, J =6.2 Hz, NH), 6.45 (1H, br t, NH), 6.82 (2H, d, 9 Hz, 2 x ArH), 7.11 (1H,

s, imidazolone 5-H), 7.87 – 8.00 (6H, m, 6 x ArH), and 8.41 (1H, br t, NH); m/z 969 (M-1)$^-$.

**Preparation of N-3-(4-trifluoroacetamidobutanoyl)aminopropyl 1-(2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (24)**

N-(3-(4-trifluoroacetamidobutanoyl)aminopropyl) 1-(3,'5'-di(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (440 mg, 0.62 mmol) and ammonium fluoride (137 mg, 3.7 mmol) were heated together at reflux in methanol (20 ml) for 48 hours. The solvent was evaporated and the residue chromatographed and the product eluted in 15% methanol in dichloromethane as a colourless solid (125 mg, 42%). $\delta_H$ ($CD_3OD$) 1.70 (2H, quintet, J = 6.8 Hz, $NHCH_2CH_2CH_2$), 1.81 (2H, quintet, J = 7.2 Hz, $NHCH_2CH_2CH_2CO$), 2.21 (2H, t, J = 7.2 Hz, $NHCH_2CH_2CH_2CO$), 2.15 – 2.24 (1H, m obscured, 2'-$CH_aH_b$), 2.31 (1H, ddd, J = 13.6, 6.6, and 6.2 Hz, 2'-$CH_aH_b$), 3.18 (2H, t, J = 6.6 Hz, $CONHCH_2CH_2CH_2NH$), 3.27 (4H, m, $CF_3CONHCH_2$, $CONHCH_2CH_2CH_2NH$), 3.62 (1H, dd, J = 11.9 and 4.4 Hz, 5'-$CH_aH_b$), 3.68 (1H, dd, J = 11.9 and 3.8 Hz, 5'-$CH_aH_b$), 3.86 (1H, br d, J = 3.1 Hz, 4'-CH), 4.37 (1H, br m, 3'-CH), 5.97 (1H, t, J = 7.0 Hz, 1'-CH), 7.32 (1H, s, imidazolone 5H), and 7.99 (1H, br m, NH); $\delta_C$ ($CD_3OD$) 24.59, 28.79, 32.68, 36.30, 36.38, 38.96, 39.19, 62.10, 71.29, 82.79, 87.19, 112.29, 116.11, 117.67, 152.86, 157.60, 159.72 , and 173.90; m/z 480.30 (M-1)$^-$.

**Preparation of N-3-(4-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)-butanoyl)aminopropyl 1-(2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

This nucleoside was prepared according to the same procedure as **24** using N-(3-(4-(4-(4-dimethylaminophenyl)azobenzenesulfonamido)butanoyl)aminopropyl 1-

(3',5'-di(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (0.8 g, 0. 9 mmol) and ammonium fluoride (1.2 g, 32 mmol) in methanol (50 ml). The reaction mixture was purified by chromatography, eluting the desired nucleoside (300 mg, 50 %) in 7.5% methanol in dichloromethane as an orange oil. $\delta_H$ ($CD_3OD$) 1.63 – 1.74 (4H, m, 2 x $CH_2CH_2CH_2$), 2.19 (2H, t, J = 7.8 Hz, $CH_2CO$), 2.15 – 2.22 (2H, partly obscured m, 2'-$CH_2$), 2.87 (2H, t, J = 6.9 Hz, $CH_2SO_2$), 3.05 (6H, s, $N(CH_3)_2$) 3.14 (2H, t, J = 6.6 Hz, $CH_2N$), 3.25 (2H, m, $CH_2N$), 6.37 (2H, m, 5'-$CH_2$), 3.85 (1H, m, 4'-CH), 4.36 (1H, m, 3'-CH), 5.96 (1H, t, 1'-CH), 6.75 (2H, d, J = 7.5 Hz, 2 x ArH), 7.28 (1H, s, imidazolone 5H), 7.79 (2H, d, J = 7.5 Hz, 2 x ArH), 7.85 (2H, d, J = 9 Hz, 2 x ArH), and 7.86 (2H, d, J = 9 Hz, 2 x ArH); m/z 671.33 $(M-1)^-$.

**Preparation of N-4-(3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propylamino)-4-oxobutyl 1-(2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

This nucleoside was prepared according to the same procedure as **24** using N-(4-(3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propylamino)-4-oxobutyl 1-(3',5'-di(*tert*-butyldimethylsilyloxy)-2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (500 mg, 0.55 mmol) and ammonium fluoride (200 mg, 11.2 mmol) in methanol (20 ml). The reaction mixture was purified by chromatography, eluting the desired nucleoside (0.18 g, 49%) in 10% methanol in dichloromethane as an orange solid. $\delta_H$ ($CD_3OD$) 1.64 (2H, quintet, J = 6.9 Hz, $NHCH_2CH_2CH_2N$), 1.78 (2H, quintet, J = 7.2 Hz, $CH_2CH_2CH_2CO$), 2.10 – 2.22 (4H, m, $CH_2CO$, 2'-$CH_2$), 2.91 (2H, t, J = 6.9 Hz, $CH_2NHSO_2$), 3.10 (6H, s, $(CH_3)_2N$), 3.16 – 3.31 (4H, m, 2 x $CH_2NHCO$), 3.67 (2H, m, 5'-$CH_2OH$), 3.88 (1H, m, 4'-CH), 4.39 (1H, m, 3'-CH), 5.98 (1H, t, J = 6.2 Hz, 1'-CH), 6.82 (2H, d, J = 9.6 Hz, 2 x ArH), 7.29

(1H, s, imidazolone 5-H), 7.84 (2H, d, J = 9.6 Hz, 2 x ArH), and 7.92 (4H, m, $N_2C_6H_4SO_2$); m/z 673.23 $(M+1)^+$.

**Preparation of N-3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propyl 1-(2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-4-carboxamide**

This was prepared according to the same procedure as **24** using N-3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propyl 1-(2'-deoxy-3',5'-di(*tert*-butyldimethylsilyloxy)ribos-1'-yl)imidazolidin-2(3*H*)-4-carboxamide (280 mg) and ammonium fluoride (240 mg, 6.5 mmol) in methanol (20 ml).The desired nucleoside (110 mg, 0.19 mmol) was isolated as an orange solid by chromatography, eluting in 10% methanol in dichloromethane. $\delta_H$ ($CD_3OD$) 1.72 (2H, quintet, J = 6.9 Hz, $NCH_2CH_2CH_2N$), 2.23 (1H, m, 2'-$CH_aH_b$), 2.30 (1H, m, 2'-$CH_aH_b$), 2.94 (2H, t, J = 6.9Hz, $CH_2NHSO_2$), 3.30 (2H, obscured m, $CONHCH_2$), 3.67 (2H, m, 5'-$CH_2$), 3.88 (1H, m, 4'-CH); 4.38 (1H, m, 3'-CH), 5.97 (1H, t, J = 6.9 Hz, 1'-CH), 6.84 (2H, d, J = 9 Hz, 2 x ArH), 7.28 (1H, s, imidazolone 5-H), 7.87 (2H, d, J = 9 Hz, 2 x ArH), and 7.90 – 7.96 (4H, m, $N_2C_6H_4SO_2$).

**Preparation of N-6-(6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanamido)hexyl 1-(2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

This was prepared according to the same procedure **24** using N-(6-(6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanamido)hexyl 1-(2'-deoxy-3',5'-di(*tert*-butyldimethylsilyloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (720 mg) and ammonium fluoride (240 mg, 6 mmol) in methanol (20 ml). The desired nucleoside (260 mg, 32 % over two steps) was isolated as an orange solid by chromatography, eluting in 10 %

methanol in dichloromethane. $\delta_H$ ($D_6$-DMSO) 1.10 – 1.42 (14 H, m, 7 x $C\underline{H}_2$), 1.97 (2H, t, J = 7.2 Hz, $C\underline{H}_2CO$), 2.09 (2H, m, 2'-$C\underline{H}_2$), 2.73 (2H, t, J = 6.9 Hz, $C\underline{H}_2NHSO_2$), 2.96 (2H, br t, $C\underline{H}_2NH$), 3.08 (6H, s, $(C\underline{H}_3)_2N$), 3.13 (2H, partially obscured m, $C\underline{H}_2NH$), 3.44 (2H, m, 5'-$C\underline{H}_2$), 3.71 (1H, m, 4'-C$\underline{H}$), 4.21 (1H, m, 3'-C$\underline{H}$), 4.85 (1H, t, 6 Hz, 5'-O$\underline{H}$), 5.20 (1H, d, J = 6 Hz, 3-O$\underline{H}$), 5.82 (1H, t, J = 6.3 Hz, 1'-C$\underline{H}$), 6.85 (2H, d, J = 9.3 Hz, 2 x Ar$\underline{H}$), 7.25 (1H, s, imidazolone 5$\underline{H}$), 7.82 (2H, d, J = 9.3 Hz, 2 x Ar$\underline{H}$), and 7.89 (4H, s, $N_2C_6\underline{H}_4SO_2$); $\delta_C$ ($D_6$-DMSO) 26.19, 27.10, 27.44, 30.11, 30.45, 36.56, 36.61, 39.51, 39.60, 43.75, 63.30, 72.05, 82.81, 88.20, 112.95, 113.03, 118.65, 123.54, 126.73, 129.12, 143.97, 153.36, 154.47, 155.81, 159.65, and 172.00; m/z 743.14 ($M^+$).

**Preparation of N-3-(4-trifluoroacetamidobutanoyl)aminopropyl 1-(2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (25)**

N-(3-(4-trifluoroacetamidobutanoyl)aminopropyl) 1-(2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (110 mg, 0.23 mmol) in dry pyridine (2 ml) was treated with 4,4'-dimethoxytrityl chloride (93 mg, 0.27 mmol) in dichloromethane (1 ml) and 4-N,N-dimethylaminopyridine (10 mg). The mixture was stirred at room temperature for 6 hours. Portions of dimethoxytrityl chloride were added until tlc (9:1 dichloromethane:methanol) showed that no starting material remained. The solvent was removed *in vacuo* and the desired product isolated by chromatography eluting with 100:10:1 dichloromethane:methanol:triethylamine to give the trityl ether (180 mg, 80%) as a colourless solid accompanied by 2 molar equivalents of triethylamine. $\delta_H$ ($CD_3CN$) 1.60 (2H, br quintet, J = 6.3 Hz, $NCH_2C\underline{H}_2CH_2N$), 1.76 (2H, quintet, J = 6.6 Hz, $CH_2C\underline{H}_2CH_2CO$), 2.20 (2H, t, J = 6.9 Hz, $CH_2CH_2C\underline{H}_2CO$), 2.15 – 2.60 (2H, partially obscured m, 2-$C\underline{H}_2$), 3.14 (4H, m, 2 x $C\underline{H}_2N$), 3.23 (4H, m, $C\underline{H}_2N$, 5'-$C\underline{H}_2$), 3.70 (6H, s, 2 x $C\underline{H}_3O$), 3.89 (1H, br m, 4'-C$\underline{H}$), 4.35 (1H, br m, 3'-C$\underline{H}$), 5.94 (1H, t, J = 6.6

Hz), 6.80 (4H, d, J = 8.4 Hz, 4 x ArH), 7.12 (1H, s, imidazolone 5H), 7.15 – 7.24 (3H, m, 3 x ArH), 7.29 (4H, d, J = 8.4 Hz, 2 x ArH), 7.40 (2H, d, J = 7.2 Hz, 2 x ArH), and 7.55 (1H, br m, $CF_3CONH$); $\delta_C$ ($CD_3CN$) 25.07, 29.85, 33.96, 37.15, 39.92, 40.24, 55.73, 65.21, 71.96, 82.91, 86.30, 86.84, 113.22, 113.91, 117.10, 118.20, 119.09, 127.63, 128.69, 128.90, 130.85, 130.89, 136.80, 136.85, 146.03, 153.73, 157.73, 159.46, 159.99, and 173.93; m/z 782.22 $(M-1)^-$.

**Preparation of N-3-(4-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)-butanoyl)aminopropyl 1-(2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

This was prepared in the same manner as **25** using N-(3-(4-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)-butanoyl)aminopropyl 1-(2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (300 mg, 0.48 mmol) in anhydrous pyridine (2 ml) and 4,4'-dimethoxytrityl chloride (134 mg, 0.4 mmol) in dichloromethane (4 ml). A further portion of 4,4'-dimethoxytrityl chloride (200 mg) was added. The desired material was isolated by chromatography in dichloromethane:methanol:triethylamine (100:10:1) to give 330 mg (74%) of an orange oil contaminated with 1 molar equivalent of triethylamine; $\delta_H$ ($CD_3OD/CD_3CN$) 1.62 (2H, quintet, J = 6.6 Hz, $NHCH_2CH_2CH_2NH$), 1.75 (2H, quintet, J = 6.9 Hz, $NHCH_2CH_2CH_2CO$), 2.20 (2H, t, J = 6.9 Hz, $NHCH_2CH_2CH_2CO$), 2.29 (2H, m, 2'-$CH_2$), 2.90 (2H, t, J = 6.9 Hz, $CH_2NHSO_2$), 3.10 (6H, s, $(CH_3)_2N$), 3.10 (2H, obscured m, $CH_2NHCO$), 3.20 – 3.25 (4 H, m, 5'-$CH_2O$, $CH_2NHCO$), 3.74 (6 H, s, 2 x $CH_3O$), 3.95 (1 H, m, 4'-CH), 4.38 (1H, m, 3'-CH), 5.99 (1H, t, J = 6.3 Hz, 1'-CH), 6.81 (6 H, m, 6 x ArH), 7.16 (1H, s, imidazolone 5H), 7.19 – 7.30 (9 H, m, 9 x ArH), 7.41 (2H, d, J = 8.4 Hz, 2 x ArH), 7.84 (4H, d, J = 9 Hz, 4 x ArH), and 7.90 (4H, m, N-$C_6H_4$-$SO_2$); $\delta_C$ ($CD_3OD/CD_3CN$) 19.14, 22.58, 26.33, 30.00,

32.75, 48.13, 57.93, 64.99, 76.07, 79.41, 79.91, 105.02, 105.74, 106.51, 110.56, 115.86, 119.07, 120.22, 123.66, 129.56, 133.82, 138.75, 147.28, 149.17, 152.46, 153.35, and 167.66; m/z 975.11 ($M^+$).

**Preparation of N-4-(3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propylamino)-4-oxobutyl 1-(2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

This was prepared in the same manner as **25** using N-(4-(3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propylamino)-4-oxobutyl 1-(2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (170 mg, 0.25 mmol) in anhydrous pyridine (2 ml) and 4,4'-dimethoxytrityl chloride (134 mg, 0.4 mmol) in dichloromethane (4 ml). A further two portions of 4,4'-dimethoxytrityl chloride (40 and 36 mg respectively) were added. The desired material was isolated by chromatography in dichloromethane:methanol:triethylamine (100:4:1) and the gum triturated in ether to give the desired material (139 mg, 57%) as an orange solid. $\delta_H$ ($D_6$-DMSO) 1.51 (2H, quintet, J = 6.9 Hz, $NHCH_2C\underline{H}_2CH_2NH$), 1.63 (2H, quintet, J = 6.9 Hz, $NHCH_2C\underline{H}_2CH_2CO$), 2.05 (2H, t, J = 6.9 Hz, $NHCH_2CH_2C\underline{H}_2CO$), 2.14 (2H, m, 2'-$C\underline{H}_2$), 2.78 (2H, br t, $C\underline{H}_2NHSO_2$), 3.00 (2H, partially obscured m, $C\underline{H}_2N$), 3.11 (6H, s, $(C\underline{H}_3)_2N$), 3.15 (2H, m, $CH_2N$), 3.35 (2H, obscured m, 5'-$C\underline{H}_2$), 3.71 (6H, s, 2 x $C\underline{H}_3O$), 3.81 (1H, m, 4'-C$\underline{H}$), 4.15 (1H, m, 3'-C$\underline{H}$), 5.30 (1H, br s, 3-O$\underline{H}$), 5.84 (1H, t, J = 6.3 Hz, 1'-C$\underline{H}$), 6.82 – 6.86 (6 H, m, 6 x Ar$\underline{H}$), 7.18 – 7.29 (7H, m, 7 x Ar$\underline{H}$), 7.35 (1H, s, imidazolone 5$\underline{H}$), and 7.80 – 7.90 (8H, m, 8 x Ar$\underline{H}$); m/z 975.08 ($M^+$).

**Preparation f N-3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propyl 1-(2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

This compound was prepared according to the procedure used for preparing **25** using N-3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propyl 1-(2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-4-carboxamide (110 mg, 0.19 mmol) in anhydrous pyridine (2 ml) and 4,4'-dimethoxytrityl chloride (79 mg, 0.23 mmol) in dichloromethane (4 ml). Further aliquots of 4,4'-dimethoxytrityl chloride (20, 16,13 and 13 mg) were added. The desired material (99 mg, 58 %) was isolated after chromatography in dichloromethane:methanol:triethylamine (100:10:1). $\delta_H$ ($D_6$-DMSO) 1.58 (2H, quintet, J = 6.9 Hz, $NHCH_2C\underline{H}_2CH_2N$), 2.13 (2H, m, 2'-$C\underline{H}_2$), 2.80 (2H, br t, $C\underline{H}_2NHSO_2$), 3.08 (6H, s, $(C\underline{H}_3)_2N$), 3.15 (2H, partially obscured m, $C\underline{H}_2NHCO$), 3.30 (2H, obscured m, 5'-$C\underline{H}_2$), 3.79 (1H, m, 4'-C<u>H</u>), 4.17 (1H, m, 3'-C<u>H</u>), 5.30 (1H, d, 3'-O<u>H</u>), 5.81 (1H, t, J = 6.2 Hz, 1'-C<u>H</u>), 6.83 (6H, m, 6 x $C\underline{H}_2$), 7.17 – 7.27 (8H, m, 8 x $C\underline{H}_2$), 7.82 (2H, d, J = 9.3 Hz, 2 x Ar<u>H</u>), and 7.89 (4H, s, $N_2C_6\underline{H}_4SO_2$); m/z 890 ($M^+$).

**Preparation of N-(6-(6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanamido)hexyl 1-(2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide**

This was prepared according the procedure used for the preparation of **25** using N-6-(6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanamido)hexyl 1-(2'-deoxyribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (200 mg, 0.27 mmol) in anhydrous pyridine (2 ml) and 4,4'-dimethoxytrityl chloride (110 mg, 0.32 mmol) in dichloromethane (4 ml). Further aliquots of 4,4'-

dimethoxytrityl chloride (36, 35, 25, 23, and 20 mg). Chromatography eluted the desired material (220 mg, 75 %) in dichloromethane:methanol:triethylamine (100:5:1) contaminated with triethylamine, and methanol. $\delta_H$ ($D_6$-DMSO) 1.12 – 1.45 (14 H, m, 7 x $CH_2$), 1.97 (2 H, t, J = 7.2 Hz, $CH_2CO$), 2.08 – 2.20 (2 H, m, 2'-$CH_2$), 2.70 (2 H, t, J = 6.9 Hz, $CH_2SO_2$), 2.96 (2 H, m, $CH_2NH$), 3.09 (2 H, obscured m, $CH_2NH$), 3.36 (2 H, obscured m, 5'-$CH_2$), 3.70 (3 H, s, $CH_3O$), 3.71 (3 H, s, $CH_3O$), 3.80 (1 H, m, 4'-CH), 4.17 (1 H, m, 3'CH), 5.31 (1 H, d, 3'-OH), 5.83 (1 H, t, J = 6.3 Hz, 1'-CH), 6.83 (6 H, m, 6 x ArH), 7.17 – 7.28 (8H, m, 8 x ArH), 7.37 (2H, d, J = 7.2 Hz, 2 x ArH), 7.82 (2H, d, J = 9.3 Hz, 2 x ArH), and 7.89 (4 H, s, $N_2C_6H_4SO_2$); m/z 1045.07 ($M^+$).

**Preparation of N-3-(4-trifluoroacetamidobutanoyl)aminopropyl 1-(3'-(2-cyanoethyl)diisopropylaminophosphityl-2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (26)**

N-(3-(4-trifluoroacetamidobutanoyl)aminopropyl) 1-(2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (180 mg, 0.18 mmol) was dissolved in anhydrous tetrahydrofuran (3 ml) under an atmosphere of argon. The solution was treated with N,N-diisopropylethylamine (71 mg, 0.55 mmol) and then (2-cyanoethyl)diisopropylchlorophosphoramidite (65 mg, 0.27 mmol) with ice cooling. The mixture was stirred at room temperature. After 10 minutes, tlc (9:1 dichloromethane:methanol) showed starting materials remained so a further aliquot of N,N-diisopropylethylamine (48 mg, 0.36 mmol) and (2-cyanoethyl)diisopropylchlorophosphoramidite (40 mg, 0.18 mmol) were added. After 30 minutes tlc showed no starting material remained. Ethyl acetate (25 ml) was added to the reaction and the solution was washed with brine (10 ml), dried ($MgSO_4$), filtered and evaporated. The resultant oil was taken into dichloromethane (1.5 ml) and added to stirred ice-cold 40-

60 petroleum ether to precipitate the solid. The product was dried *in vacuo* and used without further purification. $\delta_P$ ($CD_3CN$) 147.95, and 148.17.

**Preparation of N-(3-(4-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)-butanoyl)aminopropyl 1-(3'-(2-cyanoethyl)diisopropylaminophosphityloxy-2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (27)**

This phosphoramidite was prepared according to the same procedure as **26** using N-(3-(4-(4-(4-dimethylaminophenyl)azobenzenesulfonamido)-butanoyl)aminopropyl 1-(2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (138 mg, 0.14 mmol) and (2-cyanoethyl)diisopropylaminochlorophosphoramidite (50 mg, 0.21 mmol) and diisopropylethylamine (54 mg, 0.42 mmol). Further aliquots of (2-cyanoethyl)diisopropylaminochlorophosphoramidite (50 mg) and diisopropylethylamine (54 mg) were required to ensure that the reaction went to completion. The product was obtained as an orange solid (150 mg) following precipitation. $\delta_P$ ($CD_3CN$) 148.04, 148.27.

**Preparation of N-(4-(3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propylamino)-4-oxobutyl 1-(3'-(2-cyanoethyl)diisopropylaminophosphityloxy)-2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (28)**

This phosphoramidite was prepared according to the same procedure as **26** using N-(4-(3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propylamino)-4-oxobutyryl 1-(2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (139 mg, 0.14 mmol) and (2-

cyanoethyl)diisopropylaminochlorophosphoramidite (50 mg, 0.21 mmol) and diisopropylethylamine (54 mg, 0.42 mmol). Further aliquots of (2-cyanoethyl)diisopropylaminochlorophosphoramidite (50 mg) and diisopropylethylamine (54 mg) were required to ensure that the reaction went to completion. The product was obtained as an orange solid (139 mg) following precipitation. $\delta_P$ ($CD_3CN$) 147.98 and 148.22.

**Preparation of N-6-(6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanamido)hexyl 1-(3'-(2-cyanoethyldiisopropylaminophosphityloxy)-2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (29)**

This phosphoramidite was prepared according to the same procedure as **26** using N-(6-(6-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)hexanamido)hexyl 1-(2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (111 mg, 0.11 mmol), (2-cyanoethyl)diisopropylaminochlorophosphoramidite (38 mg, 0.16 mmol), and diisopropylethylamine (41 mg, 0.32 mmol). Further aliquots of (2-cyanoethyl)diisopropylaminochlorophosphoramidite (38 mg, 0.16 mmol) and diisopropylethylamine (52 mg, 0.32 mmol) to ensure that the reaction had gone to completion. The product was obtained as an orange solid (105 mg) following precipitation. $\delta_P$ ($CD_3CN$) 148.05 and 148.26.

**Preparation of N-3-(4-(4-N,N-dimethylaminophenyl)azobenz nesulf namido)propyl 1-(3'-(2-cyanoethyl)diisopropylaminophosphityloxy)-2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (30)**

This phosphoramidite was prepared according to the same procedure as **26** using N-3-(4-(4-N,N-dimethylaminophenyl)azobenzenesulfonamido)propyl 1-(2'-deoxy-5'-(4,4'-dimethoxytrityloxy)ribos-1'-yl)imidazolidin-2(3*H*)-one-4-carboxamide (80 mg, 0.09 mmol), (2-cyanoethyl)diisopropylaminochlorophosphoramidite (43 mg, 0.18 mmol), and diisopropylethylamine (47 mg, 0.36 mmol). Further aliquots of (2-cyanoethyl)diisopropylaminochlorophosphoramidite (43 mg, 0.18 mmol) and diisopropylethylamine (47 mg, 0.36 mmol) were added to ensure that the reaction had gone to completion. The product was obtained as an orange solid (75 mg) following precipitation. $\delta_P$ ($CD_3CN$) 147.99 and 148.23.

**EXAMPLE 4**

**Preparation of Templates containing imidazolidin-2(3*H*)-one-4-carboxamide base analogues**

Five oligonucleotides were prepared, each of the same sequence, differing only in the imidazolidin-2(3*H*)-one-4-carboxamide phosphoramidite used to synthesise them.

**Oligonucleotide sequence**

5'- ACT GXA AGG GGA TCC TCT AGA GTC GAC CTG CA –3'

where X is the imidazolidin-2(3*H*)-one-4-carboxamide nucleotide

**Template 1** : synthesised using phosphoramidite **26**
**Template 2** : synthesised using phosphoramidite **27**
**Template 3** : synthesised using phosphoramidite **28**
**Template 4** : synthesised using phosphoramidite **29**
**Template 5** : synthesised using phosphoramidite **30**

The phosphoramidites prepared as described above were dissolved in DNA reagent grade acetonitrile supplied by Cruachem to make 1 mmolar solutions. The natural base phosphoramidites were obtained from Amersham Pharmacia Biotech and dissolved in DNA grade acetonitrile according to the manufacturer's instructions immediately before oligonucleotide synthesis. The synthetic phosphoramidites were dissolved in DNA reagent grade acetonitrile to make 1 mmolar solutions with the exception of **29**, which was dissolved in 1:1 tetrahydrofuran:acetonitrile

The oligonucleotides were synthesised in three steps on an ABI 394 DNA synthesiser.

The first 27 bases were synthesised using the preprogammed 0.2 μM CE cycle (DMT On) on a 1000A CPG A column from ABI.

In the next step, the imidazolidin-2(3*H*)-one-4-carboxamide base was added using a manual cycle (DMT On) synthetic phosphoramidite (**26**) was reacted using this cycle with a coupling time of 6 minutes and the DABSYL labelled analogues (**27 - 30**) was reacted with a coupling time of 12 minutes.

Finally, the last four bases were added using the pre-programmed 0.2 μM CE cycle (DMT Off).

The oligonucleotides were cleaved from the CPG support using ammonia and the bases deprotected by heating the ammoniacal solution at 57°C for 18 hours. The crude oligonucleotides were PAGE purified and desalted using a NAP-5 column using water as eluent.

## Read-through Experiments

Recognition of the imidazolidin-2(3*H*)-one-4-carboxamide nucleobase analogues in oligonucleotides by polymerase enzymes was carried out in the following read-through experiments.

The oligonucleotides described above were used as templates and a 25 mer primer (5'-FAM-TGC AGG TCG ACT CTA GAG GAT CCC C-3') (supplied by Oswell)

## EXAMPLE 4A

A hybridisation mixture consisting of primer (7 µl of a 46 µM solution in water), **Template 1** (7 µl of a 100 µM solution in water), 5 X KGB buffer (250 mM Tris acetate, 17.5 mM magnesium acetate, 125 mM potassium glutamic acid, 10% glycerol, pH 7.9) (28 µl) and double distilled water (28 µl) was heated to 95°C for 10 minutes and cooled slowly to room temperature.

A premix of Thermosequenase II™ (20U, 5 µl), dNTP (5 µl of an 8 mM solution) and water (40 µl) was prepared.

The hybridisation mixture (50 µl) and premix were mixed and the reaction mixture incubated at 72°C and 10 µl samples were taken before incubation and after 30, 60, 90, 120, 150, 180, 210, 240, and 600 seconds. The reaction samples were quenched with EDTA (2 µl of a 50 mM solution at pH 8). Orange G in 80% formamide (5 µl) was added and the mixture heated to 95°C for 3 minutes. Primer alone, primer plus template, primer plus template plus enzyme, primer plus enzyme plus dNTPs, template plus enzyme plus dNTPs, primer plus template plus dNTPs controls were also carried out, incubating at 72°C for 300 s.

The reactions were analysed on 8% denaturing polyacrylamide gel and imaged on a Molecular Dynamics Fluorimager. This

showed that full length extended primer was form d in the reaction and therefore that Thermosequenase II™ had read through the base analogue in the template oligonucleotide.

**EXAMPLE 4B**

A hybridisation mixture consisting of primer (7 µl of a 46 µM solution in water), **Template 2** (7 µl of a 100 µM solution in water), 5 X KGB buffer (250 mM Tris acetate, 17.5 mM magnesium acetate, 125 mM potassium glutamic acid, 10% glycerol, pH 7.9) (28 µl) and double distilled water (28 µl) was heated to 95°C for 10 minutes and cooled slowly to room temperature.

A premix of Thermosequenase II™ (20U, 5 µl), dNTP (5 µl of an 8 mM solution) and water (40 µl) was prepared.

The hybridisation mixture (50 µl) and premix were mixed and the reaction mixture incubated at 72°C and 10 µl samples were taken before incubation and after 30, 60, 90, 120, 150, 180, 210, 240, and 600 seconds. The reaction samples were quenched with EDTA (2 µl of a 50 mM solution at pH 8). Orange G in 80% formamide (5 µl) was added and the mixture heated to 95°C for 3 minutes. Primer alone, primer plus template, primer plus template plus enzyme, primer plus enzyme plus dNTPs, template plus enzyme plus dNTPs, primer plus template plus dNTPs controls were also carried out, incubating at 72°C for 300 s.

The reactions were analysed on 8% denaturing polyacrylamide gel and imaged on a Molecular Dynamics Fluorimager. This showed that full length extended primer was formed in the reaction and therefore that Thermosequenase II™ had read through the base analogue in the template oligonucleotide.

**Example 4C**

A hybridisation mixture consisting of primer (7 µl of a 46 □M solution in water), **Template 3** (7 µl of a 100 µM solution in water), 5 X KGB buffer (250 mM Tris acetate, 17.5 mM magnesium acetate, 125 mM potassium glutamic acid, 10% glycerol, pH 7.9) (28 µl) and double distilled water (28 µl) was heated to 95°C for 10 minutes and cooled slowly to room temperature.

A premix of Thermosequenase II™ (20U, 5 µl), dNTP (5 µl of an 8 mM solution) and water (40 µl) was prepared.

The hybridisation mixture (50 µl) and premix were mixed and the reaction mixture incubated at 72°C and 10 µl samples were taken before incubation and after 30, 60, 90, 120, 150, 180, 210, 240, and 600 seconds. The reaction samples were quenched with EDTA (2 µl of a 50 mM solution at pH 8). Orange G in 80% formamide (5 µl) was added and the mixture heated to 95°C for 3 minutes. Primer alone, primer plus template, primer plus template plus enzyme, primer plus enzyme plus dNTPs, template plus enzyme plus dNTPs, primer plus template plus dNTPs controls were also carried out, incubating at 72°C for 300 s.

The reactions were analysed on 8% denaturing polyacrylamide gel and imaged on a Molecular Dynamics Fluorimager. This showed that full length extended primer was formed in the reaction and therefore that Thermosequenase II™ had read through the base analogue in the template oligonucleotide.

**EXAMPLE 4D**

A hybridisation mixture consisting of primer (7 µl of a 46 µM solution in water), **Template 4** (7 µl of a 100 µM solution in water), 5 X KGB buffer (250 mM Tris acetate, 17.5 mM magnesium acetate, 125 mM

potassium glutamic acid, 10% glycerol, pH 7.9) (28 µl) and double distilled water (28 µl) was heated to 95°C for 10 minutes and cooled slowly to room temperature.

A premix of Thermosequenase II™ (20U, 5 µl), dNTP (5 µl of an 8 mM solution) and water (40 µl) was prepared.

The hybridisation mixture (50 µl) and premix were mixed and the reaction mixture incubated at 72°C and 10 µl samples were taken before incubation and after 30, 60, 90, 120, 150, 180, 210, 240, and 600 seconds. The reaction samples were quenched with EDTA (2 µl of a 50 mM solution at pH 8). Orange G in 80% formamide (5 µl) was added and the mixture heated to 95°C for 3 minutes. Primer alone, primer plus template, primer plus template plus enzyme, primer plus enzyme plus dNTPs, template plus enzyme plus dNTPs, primer plus template plus dNTPs controls were also carried out, incubating at 72°C for 300 s.

The reactions were analysed on 8% denaturing polyacrylamide gel and imaged on a Molecular Dynamics Fluorimager. This showed that full length extended primer was formed in the reaction and therefore that Thermosequenase II™ had read through the base analogue in the template oligonucleotide.

**EXAMPLE 4E**

A hybridisation mixture consisting of primer (7 µl of a 46 µM solution in water), **Template 5** (7 µl of a 100 µM solution in water), 5 X KGB buffer (250 mM Tris acetate, 17.5 mM magnesium acetate, 125 mM potassium glutamic acid, 10% glycerol, pH 7.9) (28 µl) and double distilled water (28 µl) was heated to 95°C for 10 minutes and cooled slowly to room temperature.

A premix of Thermosequenase II™ (20U, 5 µl), dNTP (5 µl of an 8 mM solution) and water (40 µl) was prepared.

The hybridisation mixture (50 µl) and premix were mixed and the reaction mixture incubated at 72°C and 10 µl samples were taken before incubation and after 30, 60, 90, 120, 150, 180, 210, 240, and 600. The reaction samples were quenched with EDTA (2 µl of a 50 mM solution at pH 8). Orange G in 80% formamide (5 µl) was added and the mixture heated to 95°C for 3 minutes. Primer alone, primer plus template, primer plus template plus enzyme, primer plus enzyme plus dNTPs, template plus enzyme plus dNTPs, primer plus template plus dNTPs controls were also carried out, incubating at 72°C for 300 s.

The reactions were analysed on 8% denaturing polyacrylamide gel and imaged on a Molecular Dynamics Fluorimager. This showed that full length extended primer was formed in the reaction and therefore that Thermosequenase II™ had read through the base analogue in the template oligonucleotide.

**EXAMPLE 4F**

A hybridisation mixture consisting of primer (7 µl of a 46 □M solution in water), **Template 2** (7 µl of a 100 □M solution in water), 10 x Thermopol buffer (Biolabs) (21 µl) and double distilled water (35 µl) was heated to 95°C for 10 minutes and cooled slowly to room temperature.

A premix of Bst polymerase (Biolabs) (8U, 5 µl), dNTP (5 µl of an 8 mM solution) and water (40 µl) was prepared.

The hybridisation mixture (50 µl) and premix were mixed and the reaction mixture incubated at 72°C and 10 µl samples were taken before incubation and after 30, 60, 90, 120, 150, 180, 210, 240, and 600 seconds. The reaction samples were quenched with EDTA (2 µl of a 50 mM solution at pH 8). Orange G in 80% formamide (5 µl) was added and the mixture heated to 95°C for 3 minutes. Primer alone, primer plus template, primer plus template plus enzyme, primer plus enzyme plus

dNTPs, template plus enzyme plus dNTPs, primer plus template plus dNTPs controls were also carried out, incubating at 72°C for 300 s.

The reactions were analysed on 8% denaturing polyacrylamide gel and imaged on a Molecular Dynamics Fluorimager. This showed that full length extended primer was formed in the reaction and therefore that Bst Polymerase had read through the base analogue in the template oligonucleotide.

**EXAMPLE 4G**

A hybridisation mixture consisting of primer (7 µl of a 46 µM solution in water), **Template 2** (7 µl of a 100 µM solution in water), 5 X KGB buffer (250 mM Tris acetate, 17.5 mM magnesium acetate, 125 mM potassium glutamic acid, 10% glycerol, pH 7.9) (28 µl) and double distilled water (28 µl) was heated to 95°C for 10 minutes and cooled slowly to room temperature.

A premix of TTS DNA polymerase ( as described in PCT Patent Application No. PCT/US96/20225 ) (20U, 5 µl), dNTP (5 µl of an 8 mM solution) and water (40 µl) was prepared.

The hybridisation mixture (50 µl) and premix were mixed and the reaction mixture incubated at 72°C and 10 µl samples were taken before incubation and after 30, 60, 90, 120, 150, 180, 210, 240, and 600 seconds. The reaction samples were quenched with EDTA (2 µl of a 50 mM solution at pH 8). Orange G in 80% formamide (5 µl) was added and the mixture heated to 95°C for 3 minutes. Primer alone, primer plus template, primer plus template plus enzyme, primer plus enzyme plus dNTPs, template plus enzyme plus dNTPs, primer plus template plus dNTPs controls were also carried out, incubating at 72°C for 300 s.

The reactions were analysed on 8% denaturing polyacrylamide gel and imaged on a Molecular Dynamics Fluorimager. This

showed that full length extended primer was formed in the reaction and therefore that TTS had read through the base analogue in the template oligonucleotide.

## EXAMPLE 5

## Identification of Base Complement to Imidazolidin-2(3*H*)-one-4-carboxamide Base Analogue

### EXAMPLE 5A

Four reactions were set up to identify the base complement to the imidazolidin-2(3*H*)-one-4-carboxamide base analogue.

To hybridisation mixture as prepared in example 4B (5 µl) was added

i) premix of Thermosequenase II™ (1 µl), dTTP (1 µl of 8mM solution) and water (13 µl)

ii) premix of Thermosequenase II™ (1 µl), dTTP (1 µl of 8mM solution), dATP (1 µl of 8mM solution) and water (12 µl)

iii) premix of Thermosequenase II™ (1 µl), dTTP (1 µl of 8mM solution), dGTP (1 µl of 8mM solution) and water (12 µl)

iv) premix of Thermosequenase II™ (1 µl), dTTP (1 µl of 8mM solution), dCTP (1 µl of 8mM solution) and water (12 µl)

The reactions were incubated at 72°C for 5 minutes and were quenched with EDTA (4 µl of a 50 mM solution, pH 8)

These reactions were run alongside the controls in example 4B. The reactions were analysed on 8% denaturing polyacrylamide gel and imaged on a Molecular Dynamics Fluorimager.

Only reaction ii showed product due to the primer being extended by three bases, the remaining three reactions (i, iii, and iv)

showed only the addition of two thymidine bases. These reactions show that only dATP is a bas complement to the imidazolidin-2(3*H*)-one-4-carboxamide base analogue and therefore the analogue behaves as a thymidine analogue.

**EXAMPLE 5B**

Four reactions were set up to identify the base complement to the imidazolidin-2(3*H*)-one-4-carboxamide base analogue.

To hybridisation mixture as prepared in example 4B (5 μl) was added

i) premix of Bst polymerase (1 μl), dTTP (1 μl of 8mM solution) and water (13 μl)

ii) premix of Bst polymerase (1 μl), dTTP (1 μl of 8mM solution), dATP (1 μl of 8mM solution) and water (12 μl)

iii) premix of Bst polymerase (1 μl), dTTP (1 μl of 8mM solution), dGTP (1 μl of 8mM solution) and water (12 μl)

iv) premix of Bst polymerase (1 μl), dTTP (1 μl of 8mM solution), dCTP (1 μl of 8mM solution) and water (12 μl)

The reactions were incubated at 72°C for 5 minutes and were quenched with EDTA (4 μl of a 50 mM solution, pH 8)

These reactions were run alongside the controls in example 4B. The reactions were analysed on 8% denaturing polyacrylamide gel and imaged on a Molecular Dynamics Fluorimager.

Only reaction ii showed product due to the primer being extended by three bases, the remaining three reactions (i, iii, and iv) showed only the addition of two thymidine bases. These reactions show that only dATP is a base complement to the imidazolidin-2(3*H*)-one-4-carboxamide base analogue and therefore the analogue behaves as a thymidine analogue.

**EXAMPLE 5C**

Four reactions were set up to identify the base complement to the imidazolidin-2(3*H*)-one-4-carboxamide base analogue.

To hybridisation mixture as prepared in example 2 (5 µl) was added

v) premix of TTS (1 µl), dTTP (1 µl of 8mM solution) and water (13 µl)

vi) premix of TTS (1 µl), dTTP (1 µl of 8mM solution), dATP (1 µl of 8mM solution) and water (12 µl)

vii) premix of TTS (1 µl), dTTP (1 µl of 8mM solution), dGTP (1 µl of 8mM solution) and water (12 µl)

viii) premix of TTS (1 µl), dTTP (1 µl of 8mM solution), dCTP (1 µl of 8mM solution) and water (12 µl)

The reactions were incubated at 72°C for 5 minutes and were quenched with EDTA (4 µl of a 50 mM solution, pH 8)

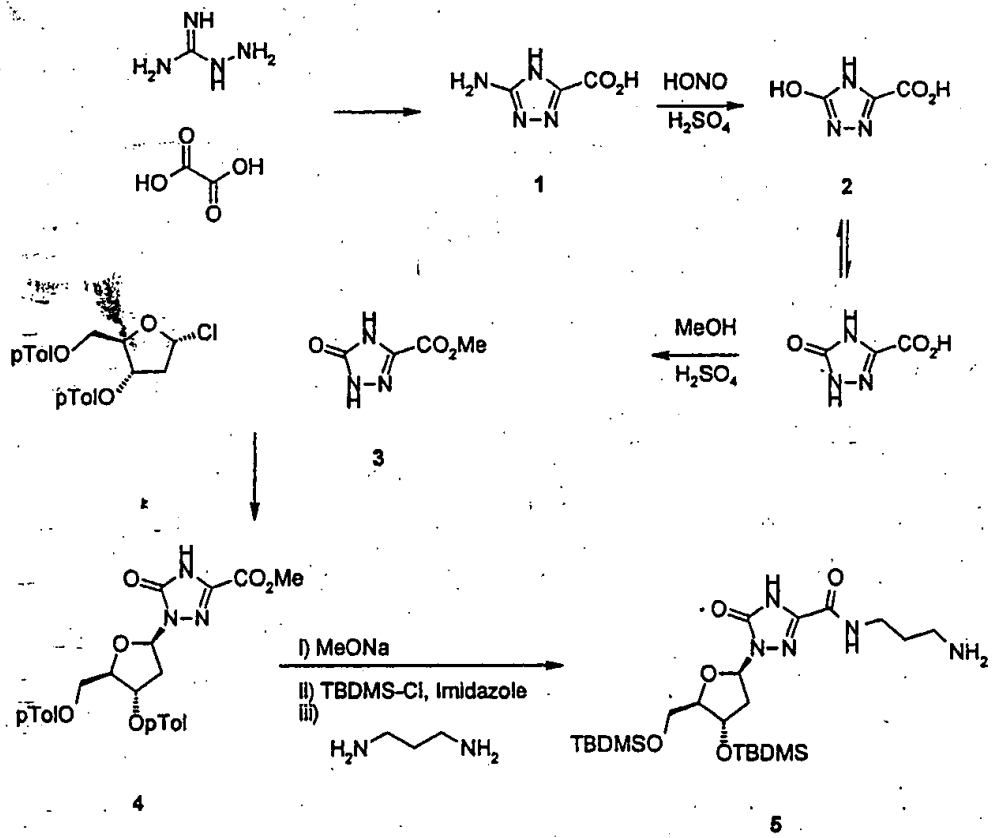
These reactions were run alongside the controls in example 2. The reactions were analysed on 8% denaturing polyacrylamide gel and imaged on a Molecular Dynamics Fluorimager.

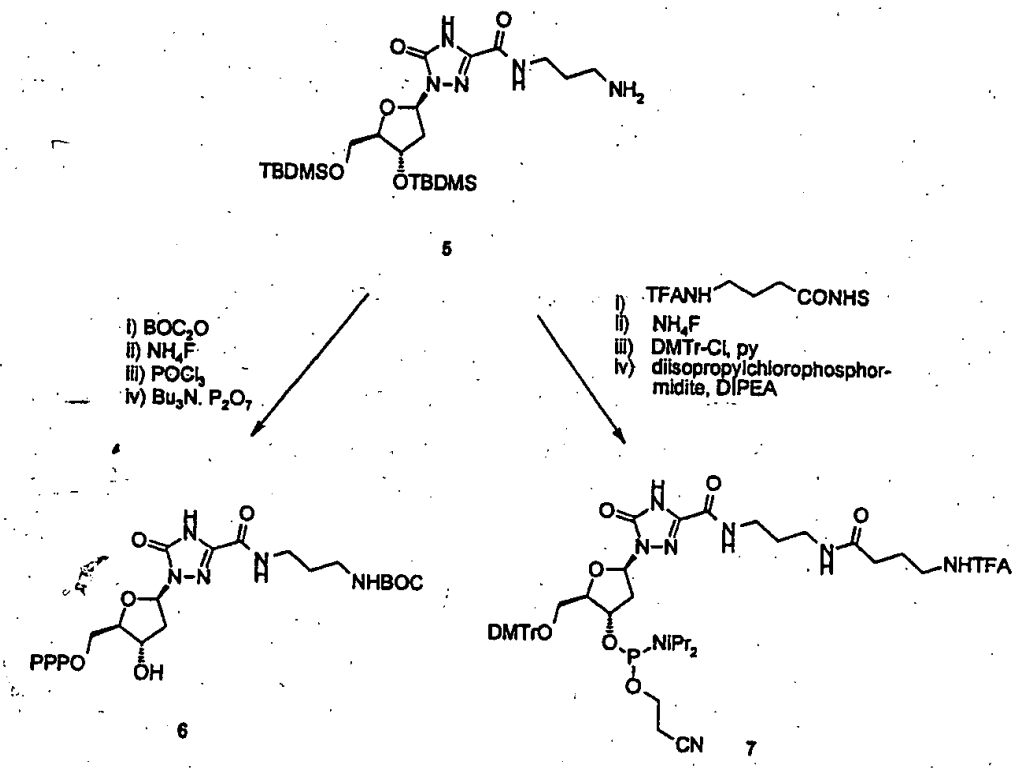
Only reaction ii showed product due to the primer being extended by three bases, the remaining three reactions (i, iii, and iv) showed only the addition of two thymidine bases. These reactions show that only dATP is a base complement to the imidazolidin-2(3*H*)-one-4-carboxamide base analogue and therefore the analogue behaves as a thymidine analogue.

**EXAMPLE 6**

Synthesis of Triazolone analogues

The synthesis of T analogue using the 1,2,4-triazol-3-one-5-carboxamides may be achieved using scheme 1. The preparation of compound 3 is known (T J Schwan and R L White, J Heterocycl. Chem., 1975, 771). This compound may be glycosylated with the 1-□-chloro-3,5-ditoluoyl-2-deoxyribose. Removal of the toluoyl protecting groups and reprotection as the silyl ethers, followed by amidation with propylenediamine gives the extended amine (5). From here the triphosphate may be prepared by protection of the amine as its *tert*-butyoxycarbamate, removal of the silyl protecting groups and phosphorylation. The phosphoramidite may be prepared by extending the amine with TFA protected 4-aminobutyric acid NHS ester, removal of the silyl protecting groups, dimethoxytritylation and phosphitylation.

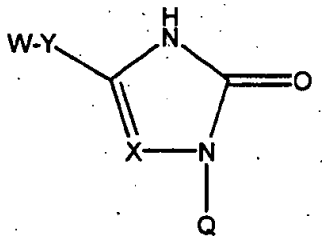





The invention is not limited to the embodiment and examples hereinbefore described which may be used in both structure and process step without departing from the invention.

## CLAIMS

1. A compound having the structure




where X is CH or N,

Y is -CO-, -CONW-, -O-, -S-, -SO-, -$SO_2$-, -NWCO-,-NW-, or -OCO-,

W is the same or different at different places in the molecule and each is H or alkyl or aryl or Rp or –Ln–Rp,

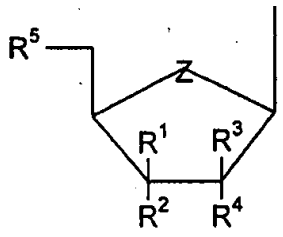
Ln is a linker group,

Rp is a reporter moiety, and

Q is a sugar or a sugar analogue or a nucleic acid backbone or backbone analogue,

provided that at least one reporter moiety Rp is present.

2. The compound as claimed in claim 1, wherein Q is




where Z is O, S, Se, SO, NW or $CH_2$,

$R^1$, $R^2$, $R^3$ and $R^4$ are the same or different and each is H,

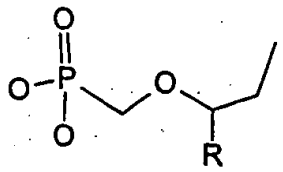
OH, F, $NH_2$, $N_3$, O-hydrocarbyl or Rp or –Ln–Rp,

$R^5$ is OH, SH or $NH_2$ or mono-, di- or tri-phosphate or -thiophosphate, or corresponding boranophosphate,

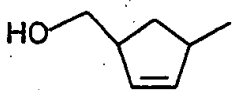
or one of $R^2$ and $R^5$ is a phosphoramidite or other group for incorporation in a polynucleotide chain, or a reporter moiety,

or Q consists of one of the following modified sugar structures

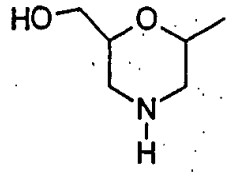
Acyclic Sugars

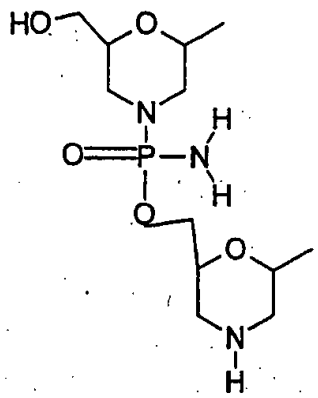


R= $CH_3$ , $CH_2OH$ , H ,



Morpholino Backbone





3. The compound of claim 1 or claim 2, wherein a reporter moiety Rp is not present in Q.

4. The compound of any one of claims 1 to 3, wherein the linker group Ln is a chain of 1 to 60 carbon, nitrogen, oxygen, phosphorus and/or sulphur atoms, rigid or flexible, saturated or unsaturated.

5. The compound of any one of claims 1 to 4, wherein the reporter moiety Rp is a signal moiety or a solid surface or a reactive group by means of which a signal moiety or a solid surface may be linked to the nucleoside or nucleotide analogue.

6. The compound of claim 5, wherein the reactive group is $NH_2$, OH, COOH, $CONH_2$, $ONH_2$, SH, or a thiophosphate or a hydrazine or a hydrazide, or an active ester or aldehyde or maleimide.

7. A nucleoside analogue comprising a compound according to any one of claims 1 to 6.

8. A nucleotide analogue comprising a compound according to any one of claims 2 to 6.

9. The nucleotide analogue of claim 8, wherein $R^5$ is triphosphate.

10. A polynucleotide chain comprising a nucleoside analogue of claim 7.

11. A polynucleotide chain according to claim 10 wherein Q is a nucleic acid backbone consisting of sugar-phosphate repeats or modified sugar-phosphate repeats (LNA), or a backbone analogue such as peptide or polyamide nucleic acid (PNA).

12. A chain extension method which comprises reacting a polynucleotide chain according to claims 10 or 11 with a primer in the presence of a polymerase.

13. A chain extension method according to claim 12 in which the primer is chosen to hybrisise with a section of the polynucleotide chain not including the nucleoside analogue.

14. A method of detecting a nucleic acid which contains a compound according to any of claims 1 to 6, which method comprises the step of detecting the presence of the reporter moiety Rp.

15. A method as claimed in claim 14 in which the reporter moiety is a radioisotope, a stable isotope, a signal moiety or a specific chemical

moiety suitable for detecting by spectroscopy, especially mass spectroscopy.